Should I Stay or Should I Go: Developmental, Physiological, Behavioural and Morphological Differences between Offspring from Alternative Life Histories

BY

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THIS THESIS IS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

Abstract

In many taxa there is considerable intraspecific variation in life-history strategies from within a single population, reflecting alternative routes through which organisms can achieve successful reproduction. Brown trout (*Salmo trutta*) and Atlantic salmon (*Salmo salar)* show some of the greatest within-population variability in life history strategies amongst vertebrates, with some individuals of a population migrating while other individuals remain resident (the phenomenon of 'partial migration'). Furthermore within each migratory ecotype, multiple discrete male and female life histories (e.g. time spent in freshwater and saltwater) co-exist and interbreed on many spawning grounds, although the effect of the various combinations of life histories on offspring traits remains unknown. These issues are covered in the General Introduction (chapter 1), which introduces the rationale for the experimental work which forms the core of this thesis.

In the first of these empirical investigations, full-sibling groups of offspring from freshwater-resident and anadromous brown trout and half-sibling groups of Atlantic salmon were reared in the laboratory under common garden conditions to examine potential differences in their early development, physiology, behaviour and phenotypic flexibility. I found that freshwater-resident parents produced eggs that were slower to hatch than did anadromous parents, but their offspring were quicker to absorb their yolk and reach the stage of exogenous feeding. Their offspring also had a higher conversion efficiency from the egg stage to the start of exogenous feeding (so were larger by the start of the fry stage) than did offspring from anadromous parents (chapter 2).

Using Atlantic salmon I showed that mothers that had spent longer at sea (two versus one year) produced offspring which were heavier, longer and in better condition at the time of first feeding. However, these relationships disappeared shortly after fry had begun feeding exogenously. At this stage, the juvenile rearing environment (i.e. time spent in fresh water) of the mother was a better predictor of offspring traits, with mothers that were faster to develop in fresh water (migrating to sea after two rather than three years of age) producing offspring that had higher maximal metabolic rates and aerobic scopes, and that grew faster. Faster developing fathers tended to produce offspring that grew faster, were in better body condition and had higher maximal metabolic rates (chapter 3). I also found that the parental type in brown trout had a significant effect on dominance status in territorial interactions, with offspring of anadromous fish being dominant over size-

matched offspring of freshwater-residents, but only when both had been raised under intermediate levels of food availability (chapter 4).

Lastly I found that offspring of anadromous trout were less able to maintain their growth rate when reared on a *Daphnia* diet than were those of freshwater-residents, and showed a correspondingly greater increase in growth following a switch in diet from *Daphnia* to Chironomid larvae. Offspring of anadromous parents also showed less morphological flexibility in response to diet than did the offspring of freshwater-residents (chapter 5). The results of these studies are summarised in the General Discussion (chapter 6), which suggests that parental effects (either direct or environmentally-mediated through differences in rearing environments) may be an important mechanism underlying the expression of threshold traits and play a significant role in the perpetuation of non-breeding partial migration and life history strategies within populations.

“The last to know about the sea is the fish”

~Chinese proverb~

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Acknowledgments

I'm truly indebted to my supervisors Profs. Colin Adams and Neil Metcalfe who have given me valuable guidance and knowledge over the last three years, I'm truly grateful. Memories of SCENE field station will live with me forever.
I would like to thank the following for the assistance that they gave:
Dr. Patrick Boylan, Dr. Ronald Campbell, Kenny Galt, members of the Tweed Foundation, Nick Chisolm, James Grubb and members of the Annan Fisheries Trust for assistance with broodstock collection; members of the Loch Lomond Fisheries Trust for help with smolt collection; Darryl McLennan, Tim Burton, David Stewart and staff from the Cromarty Firth Fisheries Trust, Graham Law, Josie Orledge and Natalie Sinclair for help with fish husbandry and measuring; Billy Fearans and Bellhaven Trout Co. for provision of facilities for early egg rearing, and Dr. Jennifer Dodd for advice on statistical analysis.

I would also like to thank the field station staff: David Fettes, Rona Brennan, Stuart Wilson and IBIS administrators: Lindsay Wilson, Andrew Smith and Hannah Mackay, my lab mates: Martin Hughes, Sinead O'Reilly, Elizabeth Clements, Nina O'Hanlon, Oliver Hooker, James Barry, Hannele Honkanen, Jessica Fordyce, Joanne Clarke, Mathew Newton and Robert Brackley for their help and insight during this project.
Lastly I would like to thank my parents Ada and Nick and brother Dustin for providing encouragement at times it was needed the most.

Author's Declaration

The material presented in this thesis is the result of research conducted between July 2012 and June 2015, under the supervision of Profs. Colin Adams and Neil Metcalfe. This work has not been submitted as part of any other degree and is based for the most part on individual research carried out by myself. In chapter 3 mating of fish and data associated with early development (prior to first feeding) was provided by Darryl McLennan. Chapter A-1 was co-written with Professor Neil Metcalfe and Dr. Shaun Killen; I was responsible for the sections in this manuscript entitled "Individual variation in metabolic responses to feeding and food availability" and "Individual variation in metabolic responses to abiotic factors".

Travis Van Leeuwen

Chapter 1: General Introduction

1.1 Animal Migration

Animal migration, the repetitive movement of hundreds or thousands of individual animals across sometimes vast geographical ranges and landscapes occurs across a wide range of taxa and has fascinated scientists for decades. Much of the work studying animal migration has highlighted the importance of migratory species in shaping ecosystem structure and function (Polis et al. 2004; Nathan 2008) but despite this, generalisations about control mechanisms and their adaptive significance remain difficult to quantify (Dodson et al 2013). This result is likely due to the considerable variation in migratory patterns exhibited between species, populations and even individuals. For example, migratory patterns between species can range from the relatively short diel vertical migrations of zooplankton and fish from the epipelagic to the mesopelagic regions of many oceans and lakes (Hansson & Hylander 2009) to the long distance seasonal movements of Monarch butterflies that migrate from North America to their overwintering grounds in Mexico and Cuba (Dockx et al. 2004). Intraspecific variation in migratory tendency has also been documented between populations. For example, European robins (*Erithacus rubecula*) from the northern part of their species range tend to exhibit a higher propensity to migrate compared to more southerly populations, which are relatively sedentary (Newton 2008). In addition to these more documented patterns of migration between species and populations, a considerable amount of variation in the propensity to migrate can occur between individuals within a population, with some individuals migrating great distances between habitats, while others remain sedentary. This phenomenon, termed 'partial migration', remains relatively understudied, compared to more traditional forms of animal migration (e.g. seasonal movements), and is the topic of this thesis.

1.2 Partial Migration

1.2.1 Definition and Types

Variation in the propensity of individuals from a single population to migrate between habitats occurs across a wide range of taxa (the phenomenon of partial migration, see reviews in Chapman et al. 2011; Dodson et al. 2013), including invertebrates (Hansson & Hylander 2009), fish (Dodson et al. 2013), birds (Newton 2008) and mammals (Ball et al. 2001). From an evolutionary perspective the maintenance of such individual variation in migratory tendency implies that the costs and benefits of migration are dependent upon context, with the net benefits not being equal for all individuals. Migration typically incurs

increased energetic costs and mortality risks, but may result in increased foraging or breeding opportunities; in the case of species with indeterminate growth this generally leads to significant growth and size-at-age benefits to migration (Jonsson & Jonsson 1993).

There are essentially three forms of partial migration: 'non-breeding', 'breeding' and 'skipped breeding' (Chapman et al. 2011). 'Non-breeding' partial migration is the commonest form of this intraspecific variation in movement patterns (Chapman et al. 2011) and occurs where migrants and residents breed sympatrically but overwinter apart. 'Breeding' partial migration occurs where migrants and residents breed allopatrically but overwinter together. Lastly 'skipped breeding' partial migration occurs where individuals migrate to breed, but not every year (Chapman et al. 2011), therefore some individuals may be considered migratory in breeding years and resident in non-breeding years. In this thesis I focus on non-breeding partial migration.

1.2.2 General Mechanisms

There have been many hypothesised explanations for the existence of non-breeding partial migration in wild populations, including, competition for resources, predation risk trade-offs and intraspecific niche diversity (see Chapman et al. 2011). In the competition for resources hypothesis, larger, dominant individuals outcompete smaller subordinate individuals for food during periods of low food availability (such as those during winter months), therefore subordinates migrate to areas with higher food availability and lower costs of territorial aggression, while larger dominant individuals remain resident, as has been demonstrated in blue tits (*Cyanistes caeruleus*) by Nilsson et al. (2008). In the predation risk trade-off hypothesis individuals with higher vulnerability to predation (i.e. bolder, smaller, more conspicuous) have a higher probability to migrate to avoid predation compared to individuals with a lower vulnerability to predation (i.e. shyer, larger, less conspicuous) as has been demonstrated in the common bream (*Abramis brama*) by Skov et al. (2011). Lastly, in the intraspecific niche diversity hypothesis, individuals that vary in their feeding niche are asymmetrically affected by seasonal changes to food availability therefore individuals from the niche which is more food limited will be more likely to migrate compared to individuals which are still able to exploit their niche food source (Chapman et al. 2011).

In all cases, however, the migration can be viewed as a response to adversity (Taylor & Taylor 1977) or as an individual response to optimise future fitness by selecting

a particular environment, but the degree of adversity and assessment of future fitness will depend on the particular environmental conditions that are experienced at the time and whether or not partial migration is fixed (i.e. influenced by the migration history of the parents) or flexible (condition-dependent). Individuals that by chance have the advantage of prior residence may have greater access to resources and so be less liable to migrate (Sandell & Smith 1991). Similarly, residency may be more likely in those encountering a low local density of competitors (as has been demonstrated experimentally in red-spotted newts (*Notophthalmus viridescens)* by Grayson & Wilbur (2009)), or those experiencing a higher food supply (as in salmonid fish: Olsson & Greenberg 2004; Wysujack et al. 2009). Body size may also affect the selection pressures for/against migration, since larger individuals may generally have less to gain from migration (Dodson et al. 2013, though see Brodersen et al., 2008 for an exception) since they usually have advantages of a higher competitive ability and/or lower risk of starvation and predation (Chapman et al. 2011).

While it is clear that both abiotic and biotic factors are likely to influence the decision to migrate or not, the relative role of genetic (fixed) versus environmental (flexible) causes remains understudied. Berthold (1998) and Berthold & Pulido (1994) provide support for a genetic pre-disposition for migratory tendency and migration distance in the Blackcap (*Sylvia atricapilla)*. However, it has also been suggested that partial migration is driven by a complex interaction between the environment and genetics: in the "threshold model" the triggering of migration depends on whether or not a continuous character ("liability trait") exceeds a genetically predetermined threshold value (Chapman et al. 2011; Dodson et al. 2013). In this scenario, at a particular time of year (a time window) individuals physiologically self-evaluate their performance against this threshold (e.g. of growth rate, body size or physiological condition), with migration being dependent on whether or not the threshold is exceeded (Fleming 1996; Thorpe & Metcalfe 1998; Pulido 2011; Dodson et al. 2013). This model generates variation in migratory tendency between environments, but can also generate partial migratory populations even under uniform conditions (since the threshold can vary between individuals); however, hard evidence of the factors that pre-dispose particular individuals to one or the other strategy is lacking.

1.2.3 Maintenance of Partial Migration Over Time

The maintenance of partial migration over evolutionary time is of great importance to evolutionary biologists and generally falls into two categories: 1) a frequency dependent

evolutionary stable state (ESS), where the fitness of the two life history strategies is equal or 2) a conditional strategy where the fitness of the individual is dependent on its phenotype (Chapman et al. 2011). Although the theoretical framework as to the maintenance of partial migration is based on the ESS hypothesis the empirical evidence in nature remains lacking due to difficulties in quantifying the fitness of individuals (Chapman et al. 2011). Although both of these strategies are similar there is one major difference. In the conditional strategy the fitness of the two life histories may not necessarily be equal and is generally determined by asymmetries of traits between individuals. For example in a population of American dippers (*Cinclus mexicanus*) migrants have a slightly higher survival rate than residents but residents have a slightly higher reproductive success but with the asymmetry between the two traits not being large enough (in terms of fitness) to drive the population toward a common life history (Chapman et al. 2011).

1.3 Brown Trout and Atlantic Salmon as Model Species

Well-documented examples of species exhibiting partial migration and/or considerable variation in life history strategies are the brown trout (*Salmo trutta)* and Atlantic salmon (*Salmo salar*). The brown trout is a polymorphic species which adopts a continuum of life history strategies, with the two most common being freshwater-resident (in which the fish carries out its entire life cycle within freshwater) and anadromous migrant (which migrates to sea, where it grows before returning to fresh water to spawn). Although several variations exist (see Behnke 1986; Etheridge et al. 2008), I focus principally on the freshwater-resident and anadromous form in this thesis, although the mechanisms described are likely applicable to the other strategies adopted by the species.

A closely related species which also shows considerable variation in life history strategies, but perhaps not as dramatic as the brown trout, is the Atlantic salmon (Fleming, 1996; Garant et al., 2003). In this species multiple discrete life histories co-exist (i.e. individuals with differing years spent in fresh and saltwater as juveniles and adults) and interbreed in many spawning areas.

During spawning time both species can be readily collected from their spawning sites by electrofishing or trapping and used as broodstock for controlled *in vitro* fertilisation experiments. Once eggs of know parentage are stripped and fertilised, eggs and offspring of known parental type can be reared under controlled simulated conditions

in the laboratory, allowing investigation of the behavioural and physiological variation that is associated with these alternative life histories. Therefore, given the diversity of life histories, the availability of these species and the potential for their eggs and offspring to be reared in the laboratory, I chose to use brown trout and Atlantic salmon for the experiments in this thesis.

1.4 Brown Trout Life History and Ecology

Indigenous to Europe, North Africa and Western Asia, brown trout have been introduced into at least twenty-four countries outside of Europe and are classed as a global species (Elliot 1994). Brown trout are iteroparous and often reproduce multiple times during their life, although this is likely less in migratory forms due to the higher energetic costs associated with migration. Mature adults of both ecotypes return to their natal freshwater stream in the winter months to spawn. Females dig a depression in the gravel where she lays a batch of eggs. Eggs are fertilised by usually one of several competing males but not necessarily of the same life history type since both ecotypes can be derived from a single gene pool, with anadromous and freshwater-resident adults having the ability to interbreed and produce offspring capable of adopting either life history (Wysujack et al. 2009; O'Neal & Stanford 2011). In populations consisting of both migratory types the migratory individuals are predominantly female with males being more likely to be freshwater-resident (Klemetsen et al. 2003). This discontinuity is likely due to differences in size-at-age benefits between males and females. For example, females gain benefits from being larger since large bodied fish tend to produce a greater number of eggs, dig deeper nests (Elliott 1984) and can excavate those nests in areas of larger gravel, all of which likely increases the survival of their young. In contrast, the reproductive benefits of a larger body size in males is less clear cut since small males can reproduce at a younger age and can obtain fertilisations by sneaking rather than fighting for access to females (Bohlin et al. 1990). Once fertilised, the eggs are covered by the female where they will remain until the following spring. Embryonic development is temperature-dependent (Elliott & Hurley, 1998) with warmer rivers producing faster developing embryos. Once hatched, embryos are known as alevins, which remain in the gravel and feed endogenously on the yolk sac provisioned by their mother prior to egg laying (Klemetsen et al. 2003). Once the yolk sac is exhausted, individuals are known as fry and emerge from the gravel and begin feeding exogenously on invertebrate drift in the water column (Klemetsen et al. 2003). At this stage salmonid fry rapidly establish size-based dominance hierarchies that are temporally stable (Jonsson & Jonsson 2010) as they engage in intra- and inter-specific

competition for preferred feeding territories (Heland 1999; Lahti et al. 2001; Klemetsen et al. 2003). It is generally accepted that dominance is advantageous in salmonid fry since it gives preferential access to food (Alanärä & Brännäs 1996) and so facilitates increased growth (Klemetsen et al. 2003); more dominant individuals tend to survive better (Huntingford & Turner 1987), since mortality rate tends to decline with fish size (Klemetsen et al. 2003), so the early phase of territorial establishment is the most critical period of the life-cycle (Elliott 1994). After a few months trout reach the parr stage and generally inhabit shallow fast-flowing microhabitats (Greenberg et al. 1996). During these stages freshwater-resident and anadromous brown trout appear morphologically indistinguishable, and it is presumed that they only become separable after one to five years (Jonsson et al. 2001) when the migrants turn silver in colour in the late winter/spring in preparation for entry to sea water in early summer (smolting; Jonsson 1985). Fish are only physiologically capable of tolerating the switch from fresh- to seawater during this smolt period in early summer, but smaller fish delay their migration until late in this window in order to maximise their size at the time of entering the sea (Bohlin et al. 1993). Freshwater-resident individuals will subsequently mature without ever leaving the freshwater environment whereas anadromous individuals will spend months or years at sea before returning to their natal freshwater stream to spawn (Klemetsen et al. 2003). Although migration distances and time spent at sea is highly variable, anadromous brown trout seem to remain relatively coastal (Klemetsen et al. 2003), with a large proportion of fish feeding in rich estuarine habitats as adults (Etheridge et al. 2008).

1.5 Atlantic Salmon Life History and Ecology

Indigenous to Europe and the Eastern coast of North America, this species has multiple discrete male and female life histories that co-exist and interbreed on many spawning grounds (Fleming 1996). Mature anadromous adults return to their natal freshwater stream in the winter months to spawn. Alternatively, some males, generally those that exhibit fast early growth (Whalen & Parrish, 1999; Aubin-Horth & Dodson, 2004), will become sexually mature at a small size without ever going to sea; these males, known as precocious male parr, participate in spawning as sneakers (Fleming, 1996). Females dig a depression in the gravel where they lay a batch of eggs. Eggs are fertilised by usually one of several competing males but not necessarily of the same life history type (Klemetsen 2003). Once fertilised, the eggs are covered by the female where they will remain until the following spring. The early development, behaviour and ecology of the Atlantic salmon is very similar to that of the brown trout. The time spent in fresh water

prior to seaward (smolt) migration ranges between 1-8 years; this variation is primarily due to variation among rivers in growth conditions, with fish migrating at a younger age in warmer rivers (Metcalfe & Thorpe, 1990), but there is also variation in age at migration within rivers (with most rivers producing at least two age classes of smolt). This variation is largely due to differences among individuals in their earlier growth rate, since the physiological decision on whether or not to migrate to sea in a given summer is largely based on size attained by the previous autumn (Metcalfe & Thorpe 1990). Once fish have undertaken the smolt migration they spend between 1-5 (most commonly 1-2) winters at sea before returning to their natal freshwater stream to spawn (Klemetsen et al. 2003). Unlike anadromous brown trout, Atlantic salmon undergo dramatic seaward migrations. For example some salmon from Western Europe move out to the open ocean in the North Atlantic, reaching Greenland, before returning to their natal freshwater stream to spawn (Klemetsen et al. 2003). Like brown trout, Atlantic salmon are potentially iteroparous and can reproduce more than once, but anadromous fish are generally in poor physiological condition after spawning and so the proportion surviving the return migration to sea is often small; in most rivers less than 20% will spawn more than once.

1.6 Potential Drivers of Partial Migration in Trout and Salmon

There have been many hypothesised explanations for the existence of alternative life history strategies in trout and salmon populations (Thorpe et al. 1998). Jonsson (1985) proposed that migrant brown trout are made up of the slower growing individuals in a population, which migrate in search of more productive habitats. Given that freshwater fluvial ecosystems are often regarded as being food limited (Imre et al.2005), it has also been suggested that metabolic constraints play an important role in determining physiological state and thus migration probability. In the low food environment of their natal river, individuals with a lower growth efficiency, higher food requirement and/or higher metabolic rate (i.e. energy maximisers) will become energetically constrained earlier in life compared to individuals with higher growth efficiency, lower food requirement and/or lower metabolic rates (efficiency maximisers; Metcalfe et al.1995; Forseth et al. 1999; Morinville & Rasmussen 2003; Rosenfeld et al. 2013). Individuals with low growth efficiency and/or higher metabolic rates may therefore migrate in search of more productive habitats (lakes, oceans) to meet their outstanding metabolic needs.

While it is likely that growth history and current body size and physiological condition influence the decision to migrate, this may in part be driven by genetic or

parental effects. Given that salmon and trout produce large yolky eggs which represent a significant nutritional source for the developing embryo, typically providing the only energy source for the first months of development, there is potential for strong parental effects on offspring development (Mousseau & Fox 1998; Monaghan 2008; Burton et al. 2011; 2013), with migration history of the parents potentially influencing the migration probability of the offspring.

1.7 Relevance of Studying Metabolism and Growth

Since fast growing juveniles tend to migrate to sea earlier than their slower growing counterparts from the same population (Metcalfe 1998), there has been interest in the intrinsic differences between fish that affect potential growth rate and hence generate life history variation. One trait which has received a considerable amount of attention is metabolic rate (Forseth et al., 1999), partly because it is often correlated with dominance or growth (Metcalfe et al., 1995; Alvarez & Nicieza, 2005) and partly because it constitutes the fundamental energy budget of organisms. Standard metabolic rate (SMR) is the minimal maintenance metabolic rate of an ectotherm in a post-absorptive and inactive state (so is the equivalent of the basal metabolic rate of an endotherm). Standard metabolic rate (usually measured in terms of oxygen consumption) is an integrated measure of the energy expenditure involved in tissue maintenance and organism homeostasis. After controlling for temperature, body size and other sources of variation, SMR often differs by a factor of 2 or 3 between individuals of the same age, sex and species held in similar conditions (Burton et al. 2011). Aerobic scope (AS) is defined as the difference between an animal's SMR and its maximum possible aerobic metabolic rate (MMR) under the same environmental conditions, so that AS defines the capacity of the animal to increase its rate of aerobic metabolism (Killen et al. 2012).

Individual differences in SMR within salmon and trout populations have been linked to variation in individual growth and life history strategies (e.g. timing of smolt migration; Metcalfe et al. 1995; Forseth et al. 1999; Finstad et al. 2007). In contrast, the role of AS remains relatively understudied, although it has been found to be correlated with swim performance, distance of migration (Eliason et al. 2011) and survival of individuals in challenging environments (Clark et al. 2011; Killen et al. 2012). However, the associations between these traits and how they may influence the life history of their offspring remains unknown.

1.8 Outline of Thesis

The main focus of this thesis is therefore to investigate the effects of migration history of the parents on offspring development, physiology, behaviour and phenotypic flexibility. The overall approach has been to rear brown trout and Atlantic salmon offspring from eggs of known parentage and then subject the fish to a number of experimental manipulations and techniques to address fundamental questions that further our understanding of potential mechanisms behind alternative life histories in partially migrating populations.

Chapter 2 discusses differences in fecundity, egg size, developmental rate, condition, growth, SMR, MMR and AS between offspring from freshwater-resident and anadromous brown trout. I test (1) the effects of migration history of the parents on egg size and number, and (2) the effects of migration history of the parents on offspring early development and metabolic rate. The results prompt the hypothesis that differences in these characteristics may be an important but overlooked mechanism underlying the expression of threshold traits and play a significant role in the perpetuation of non-breeding partial migration within populations.

Chapter 3 discusses how the duration of early and later life stages of both parents (i.e. their life history) may be a significant influence on offspring traits. Using the diversity of life history strategies in Atlantic salmon and the scope offered by conservation programmes for controlled *in vitro* fertilisations I test (1) whether there is any association between maternal or paternal life history pathway and offspring performance (i.e. growth, condition, SMR, MMR and AS), (2) whether the strength of any such effects weakens over time during offspring development, and (3) whether parents that exhibited fast juvenile growth produce offspring that themselves have higher growth and metabolic rates.

Chapter 4 discusses differences in relative dominance of offspring from alternative life histories when competing for feeding territories. Using offspring from freshwater-resident and anadromous brown trout reared on differing levels of food availability and a large swim flume I test (1) whether offspring from parents with alternative life histories differ in dominance and (2) how prior food availability influences these relationships between offspring type.

Chapter 5 discusses how offspring from parents that adopted alternative life histories respond morphologically (in terms of body shape) and physiologically (in terms of SMR and growth) to diets of differing ease of digestion. I test (1) whether offspring from freshwater-resident or anadromous parents differ in their ability to assimilate *Daphnia* and Chironomid larvae and (2) how these offspring respond morphologically (in terms of body shape) and physiologically (in terms of SMR and growth) immediately following a diet switch.

Finally Chapter 6 contains a general synthesis of all results and discusses their contribution to the understanding of partial migration.

Chapter Two: Differences in Early Developmental Rate and Yolk Conversion Efficiency in Offspring of Trout with Alternative Life Histories

*Note: A version of this chapter has been submitted as a manuscript to the journal Ecology of Freshwater Fish.

2.1 Introduction

In many taxa there is variation in the propensity of individuals from a single population to migrate between habitats (the phenomenon of 'partial migration', see reviews in Chapman et al. 2011; Dodson et al. 2013). From an evolutionary perspective the maintenance of such individual variation in migratory tendency implies that the costs and benefits of migration are dependent upon context, with the net benefits not being equal for all individuals. Migration typically incurs increased energetic costs and mortality risks, but may result in increased foraging or breeding opportunities; in the case of species with indeterminate growth, this generally leads to significant growth and size-at-age benefits to migration (Jonsson & Jonsson 1993).

The commonest form of this intraspecific variation in movement patterns is non-breeding partial migration (*sensu* Chapman et al. 2011), where migrants and residents breed in sympatry but segregate during feeding stages of their life-cycle. There have been many hypothesised explanations for this category of migration, including competition for resources or breeding opportunities, predation risk trade-offs and intraspecific niche diversity (see Chapman et al. 2011). In all cases the migration can be viewed as a response to adversity (Taylor & Taylor 1977) or as an individual response to optimise future fitness by selecting a particular environment. However, the degree of adversity and assessment of future fitness will depend on the particular environmental conditions that are experienced at the time and whether or not partial migration is fixed (i.e. influenced by the migration history of the parents) or flexible (condition-dependent). Thus individuals that by chance have the advantage of prior residence may have greater access to resources and so be less liable to migrate (Sandell & Smith 1991). Similarly, residency may be more likely in those encountering a low local density of competitors (as has been demonstrated experimentally in red-spotted newts by Grayson & Wilbur (2009), or those experiencing a higher food supply (as in salmonid fish: Olsson & Greenberg 2004; Olsson et al. 2006; Wysujack et al. 2009). Body size may also affect the selection pressures for/against migration, since larger individuals may generally have less to gain from migration (Dodson et al. 2013, though see

Brodersen et al, 2008 for an exception) since they usually have advantages of a higher competitive ability and/or lower risk of starvation and predation (Chapman et al. 2011).

While it is clear that both abiotic and biotic factors are likely to influence the decision to migrate or not, the relative role of genetic (fixed) versus environmental (flexible) causes remains understudied. Berthold (1988) and Berthold and Pulido (1994) provide support for a genetic pre-disposition for migratory tendency and migration distance in the Blackcap. However, it has also been suggested that partial migration is driven by a complex interaction between the environment and genetics: in the "threshold model" the triggering of migration depends on whether or not a continuous character ("liability trait") exceeds a genetically predetermined threshold value (Chapman et al. 2011; Dodson et al. 2013). In this scenario, individuals physiologically self-evaluate their performance against this threshold (e.g. of growth rate, body size or physiological condition), with migration being dependent on whether or not the threshold is exceeded (Fleming 1996; Thorpe & Metcalfe 1998; Pulido 2011; Dodson et al. 2013). This model generates variation in migratory tendency between environments, but also generates partial migratory populations even under uniform conditions (since the threshold can vary between individuals); however, hard evidence of the factors that pre-dispose particular individuals to one or the other strategy is lacking.

A well-documented example of a species exhibiting partial migration is the Brown trout, a polymorphic species that adopts a continuum of life history strategies, with the two most common being freshwater-resident and anadromous migrant (which grows relatively quickly at sea before returning to fresh water to spawn). Both ecotypes can occur in sympatry, possibly derived from a single gene pool, with both anadromous and freshwater-resident adults having the ability to interbreed and produce offspring capable of adopting either life history (O'Neal & Stanford 2011). Freshwater-resident and anadromous trout appear indistinguishable during early life, and it is presumed that they only become separable after one or more years, when the migrants turn silver in colour in preparation for entry to sea water (smolting; Jonsson 1985). The major physiological change that is required for the trout to enter sea water means that the option to migrate is also associated with a developmental switch-point some months prior to the actual migration (Paez et al. 2011). Jonsson (1985) proposed that migrant brown trout are made up of the slower growing individuals in a population, which migrate in search of more productive habitats. Given that freshwater fluvial ecosystems are often regarded as being food limited (Imre et

al. 2005), it has also been suggested that metabolic constraints play an important role in determining physiological state and thus migration probability. In the low food environment of their natal river, individuals with a lower growth efficiency, higher food requirement and/or higher metabolic rate (i.e. energy maximisers) will become energetically constrained earlier in life compared to individuals with higher growth efficiency, lower food requirement and/or lower metabolic rates (efficiency maximisers; Metcalfe et al.1995; Forseth et al 1999; Morinville & Rasmussen 2003; Rosenfeld et al. 2013). Individuals with low growth efficiency and/or higher metabolic rates may therefore migrate in search of more productive habitats (lakes, oceans) to meet their outstanding metabolic needs.

While it is likely that growth history and current body size and physiological condition influence the decision to migrate, this may in part be driven by genetic or parental effects. Given that trout produce large yolky eggs which represent a significant nutritional source for the developing embryo, typically providing the only energy source for the first months of development, there is potential for strong parental effects on offspring development (Mousseau & Fox 1998; Monaghan 2008; Burton et al. 2013), with migration history of the parents potentially influencing the migration probability of the offspring.

To address these issues, I reared brown trout offspring from eggs of known parentage (i.e. freshwater resident or anadromous) under common garden conditions. Because I knew the migration history of the parents, it provided an opportunity to understand (1) the effects of migration history of the parents on egg size, egg number and egg energy density and (2) the effects of migration history of the parents on offspring development. Based on the differences in adult size between the two ecotypes (with anadromous fish being considerably larger) and the metabolic constraints hypothesis for migration, we expected that offspring from anadromous parents would be larger from the egg stage through to the start of exogenous feeding, exhibit a higher metabolic rate and/or lower food conversion efficiency and consume their yolk reserves sooner, so causing them to begin feeding on exogenous food sooner than offspring of freshwater residents. Differences in early development between offspring from alternative life histories would provide evidence for parental effects as a potential mechanism underlying the expression of threshold traits and provide potential clarity as to the maintenance of alternative life histories in partially migrating populations.

2.2 Methods

2.2.1 Broodstock Collection

Twenty-four mature freshwater-resident (12 male and 12 female) and 14 anadromous (7 male and 7 female) brown trout were captured using electrofishing on 11 October 2013 and 23 October 2013 from two sub-tributaries of the River Tweed, Scotland. Freshwater-resident trout were collected from above an impassable dam on the Whiteadder River (55° 88'N, 2°57'W) while the anadromous trout were collected from the College Burn (55° 77'N, 2°18'W). Adult fish were classified as freshwater-resident or anadromous based on size and colouration (Eek & Bohlin 1997): freshwater-resident fish were smaller and dark brown in colour with red spots, while anadromous fish were larger and silvery-grey in colour with black spots. There is no history of hatchery releases into either tributary. To further reduce the risk of misclassification we collected our broodstock from two neighboring tributaries which contained sufficient numbers of males and females of known life-history type. Fish were transported to the Belhaven Trout Company, Scotland, where they were held in two round 1530 L aluminum tanks supplied with 8.1 $\pm$ 0.4 °C (mean$\pm$SD) well water under ambient photoperiod and assessed every three days for ripeness.

Ripe fish were anaesthetised, photographed, measured and blotted dry, and their eggs or sperm extruded by abdominal massage. A sample of between 9-22 unfertilised eggs from each female was collected and weighed to the nearest 0.0001g on the day of stripping (freshwater-resident n=162; anadromous n=139). These eggs were then frozen and later defrosted, re-weighed (9-10 eggs per female) and dried at 40 °C for 12 days so that relative energy density (dry mass to wet mass ratio) of individual eggs could be determined. The remaining eggs from each female were photographed (so that individual eggs could be counted at a later date to determine clutch size) and fertilised with sperm from a haphazardly-chosen male of the same life history origin to create 12 full sibling freshwater-resident families and 7 full sibling anadromous families. Freshwater-resident and anadromous fish were artificially spawned from 3 November - 29 November and 17 November - 4 December 2013 respectively.

2.2.2 Egg Rearing, Hatching and Fish Husbandry

Each family of eggs was housed separately in a plastic mesh egg basket, placed in one of two (1m X 3m X 0.4m) rearing troughs supplied with well water and covered with dark plastic sheeting to ensure eggs were in complete darkness. Water temperature during

incubation was 8.1 ± 0.4 °C and was recorded daily along with any dead eggs which were carefully removed.

Eggs were checked daily for hatching; those from freshwater-resident and anadromous fish hatched from 19 December 2013- 17 January 2014 and 30 December 2013-24 January 2014 respectively. Once eggs began to hatch, the newly emerged offspring (alevins) were counted, separated from the remaining eggs and gently placed into a small mesh basket (one per family) located in the same two troughs as the egg baskets. Ten to fifteen alevins from each family were blotted dry and weighed to the nearest 0.0001g on the day of hatching.

On 31 January 2014 alevins were transported to the Scottish Centre for Ecology and the Natural Environment, Scotland. Families were housed separately in 15 L (50cm X 30cm X 15cm) clear plastic aquaria on a partial recirculation system at a constant temperature of 9.2 ± 0.2 °C (mean±SD) and ambient photoperiod. The aquaria contained a single air stone and were supplied with water pumped directly from Loch Lomond, which was first treated with an ozone generator (Sander S1000, Germany) before being discharged into a large sump. Water from the sump was pumped through an in-line 110W UV steriliser (Tropical Marine Center (TMC), Manchester, UK) before entering the aquaria. Return water was gravity fed into a large free standing filter before being discharged back into the main sump. Fish were monitored daily and any mortalities removed. The date of first feeding for each family was determined as the point in time when the alevins' yolk sac was fully exhausted and the first individuals began to swim up from the bottom in search of food. Ten individuals from each family were anaesthetized, blotted dry and weighed to the nearest 0.0001g to determine their mass at first feeding. Additionally four fish from each family were selected at the point of first feeding for measurement of metabolic rate.

2.2.3 Measuring Maximal Metabolic Rate

Prior to SMR being measured overnight, individual fish were sequentially subjected to an exhaustive chase protocol in order to determine MMR (Killen et al. 2010; Norin 2014). A single fish was introduced into a round circular arena and hand chased for three minutes to exhaustion. The fish was then placed into 1 of 4 respirometry chambers as described below. Once a fish was placed in the chamber the flush pump was immediately turned "off" which allowed for the rate of oxygen uptake to be measured and determined

as outlined below for SMR measurements. Maximal metabolic rate was determined as the highest oxygen consumption rate over the entire 24 hour measurement period. In all cases this value corresponded to the first measurement immediately following the exhaustive chase protocol.

2.2.4 Measuring Standard Metabolic Rate

Oxygen uptake was measured over a 24 h period, from approximately 10 AM onwards, using intermittent flow respirometry. Individual fish were placed into 1 of 4 separate (6.0 cm length, 1.4cm diameter) glass respirometry chambers (Loligo systems, Tjele, Denmark) fitted with OXSP5 sensor spot (PyroScience GmbH, Aachen, Germany). Chambers were secured using a perspex micro chamber holder (Loligo systems) and submersed in a water bath housed inside a constant temperature room. An air-stone in the water bath of the respirometer apparatus kept the water fully saturated with oxygen. Water temperature averaged 9.2$\pm$ 0.2 °C across all measurements. Opaque plastic partitions prevented visual contact between individual fish during measurements, and all measurements were conducted in the dark to further minimise fish activity (Cutts et al. 2002). Glass respirometers and tygon tubing were used to prevent possible issues with use of plastics and oxygen permeable materials (Stevens 1992). Oxygen uptake was measured for 25 min every 45 min on a continuous 20 min "on" and 25 min "off" cycle. During the "on" cycle oxygenated water from the water bath was driven by a single water pump (Eheim 300 universal, Deizisau, Germany) through each of the respirometers. Flow rate was regulated by adjusting the tension of a hose clamp on the outflow side of the pump tubing to prevent swimming and spontaneous behaviour during flushing. After 20 minutes the water pump was automatically switched off (Superpro MFRT-1 timer, Somerset, England) allowing for a decrease in oxygen concentration to be measured during the 25 min "off" period, during which a peristaltic pump (Masterflex L/S, London, England) was used to ensure adequate mixing within each respirometer. Water oxygen concentration was measured every 1s for 25 min during this time period. Oxygen concentration within the respirometer was measured using an oxygen meter (FireStingO_2 oxygen meter; PyroScience) fitted with 4 bare fibre oxygen probes; concentrations never dropped below 80% oxygen saturation in this experiment. Probes were calibrated daily, and rates of background oxygen consumption were subtracted from the observed values by measuring the oxygen concentration of water inside each of the respirometers in the absence of fish at the beginning and end of each measurement trial and assuming a linear decrease in oxygen concentration over the measurement period.

Fry at the point of first feeding was used to standardise for differences in development and hatch timing between families and ecotypes and prevent potential confounding effects of differential yolk sac mass in which little metabolic activity is thought to occur (Kamler 2008; Regnier et al. 2010). The rate of oxygen consumption was determined using the following equation (Ege & Krogh 1914):

$$MO_2=V_w(\Delta C_w0_2)/\Delta t$$

where V_w is the volume of water in the respirometer and associated tubing minus the volume of the fish and ΔC_w0_2 is the change in oxygen concentration of the water over time period Δt (Steffensen 1989). Oxygen concentration was calculated by correcting PO2 (partial pressure oxygen) for barometric pressure and multiplying by αO2 (μmol L^{-1} $torr^{-1}$), the solubility coefficient at the observed temperature. Measurements of oxygen concentration were plotted graphically allowing for periods of complete rest to be readily discriminated from spontaneous activity, which appeared as distinct spikes. Standard metabolic rate was estimated by using the average of the lowest 10% of values observed during the respirometry trial (Norin 2014). Following respirometry measures all fish were anaesthetized, blotted dry and weighed to the nearest 0.0001g.

2.2.5 Calculations and Statistical Analyses

Developmental rate between ecotypes and families were compared by measuring time to reach specific stages of development (i.e. time to hatch, time to first feeding), using accumulated thermal units (ATU, days X average daily temperature) from fertilisation (Taylor et al. 2000) to standardise for slight changes in water temperature and spawning time. Survival rate was quantified as the proportion of eggs per family surviving from fertilisation to hatch. Relative egg energy density was calculated by dividing the dry mass of individual eggs by their wet mass. Conversion efficiency was calculated over three key stages of development by dividing mass at hatching by egg mass, mass at first feeding by hatch mass and mass at first feeding by egg mass. Aerobic scope was determined by subtracting SMR from MMR. Survival data were arcsine transformed while SMR, MMR, AS, female length and egg number were $\log_{10}$ transformed to linearise the data and meet assumptions of normality and homogeneity of variance.

A general linear model was used to test for the effect of female length and ecotype on clutch size, and to compare adult lengths between sex and ecotype. We used linear mixed effects models (LME) to test for the effect of female length, egg mass and ecotype on hatch time, egg survival, hatch mass, time to first feeding and mass at first feeding.

Furthermore a LME was used to test for the effect of ecotype on egg mass, egg energy density and average conversion efficiency. Lastly to test for differences in SMR, MMR and AS between ecotypes we again used an LME model but with absolute, rather than mass-specific, values of SMR, AS and MMR using mass as a fixed effect to control for differences in offspring mass. All LME models initially included all possible two way interactions and family as a random factor. Interaction terms and independent variables that were not significant at $p>0.05$ were removed from the model, which was then re-run to obtain the model of best fit. Data for both ecotypes were first analysed together. If a significant interaction with ecotype was found, data for freshwater-resident and anadromous offspring were analysed separately using the above procedure but removing ecotype as a factor. All LME analyses were conducted using the nlme function (Pinheiro et al. 2011) and R version 3.0.1 statistical software (R Core Team, 2013).

2.3 Results

2.3.1 Broodstock

As expected anadromous parental fish were larger overall at the time of spawning than freshwater-resident fish ($F_{3,34}$ =292.42, $p<0.001$; Fig. 2.1). However, there was no significant difference in length between freshwater-resident females and freshwater-resident males at the time of spawning ($F_{1,22}$ = -1.951, p=0.06; Fig. 2.1), nor between anadromous females and anadromous males at the time of spawning ($F_{1,12}$ =1.97, p=0.07; Fig. 2.1).

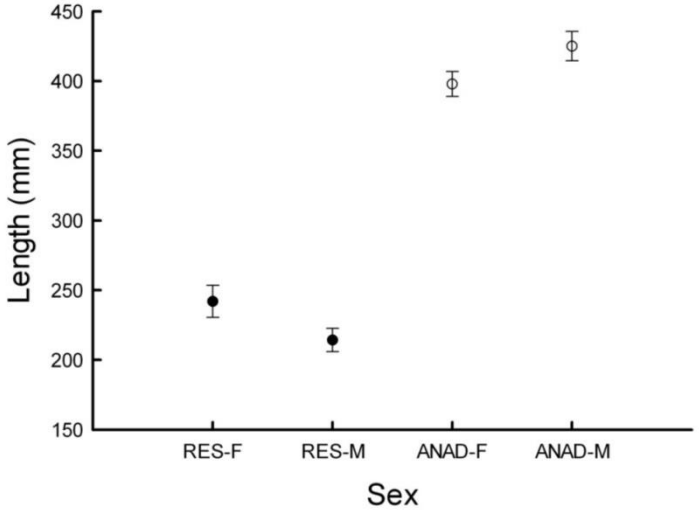

Fig. 2.1. Average length for mature female (F) and male (M) freshwater-resident (RES, closed circles) and anadromous (ANAD, open circles) brown trout spawned in this study. Error bars represent standard error of the mean. See text for statistical analysis.

2.3.2 Egg Size

Larger females produced heavier eggs ($F_{15,282}$ =63.08, p<0.001) but not necessarily a greater number of eggs, since clutch size was negatively correlated with egg mass ($F_{15,282}$=5.16, p=0.04). There was no significant difference in egg mass between eggs from anadromous females and those from freshwater-resident females (anadromous mean egg size $\pm$ SD: 0.0641 $\pm$ 0.0110 g; freshwater resident: 0.0611 $\pm$ 0.0150g $F_{17,282}$= 0.2486, p=0.6244). However, after controlling for differences in maternal body size, anadromous females produced a greater number ($F_{2,16}$=-3.243, p=0.005) of smaller eggs ($F_{15,282}$=10.31, p=0.006) than did freshwater-residents (Fig. 2.2). I found no significant difference in the energy density of eggs from anadromous and freshwater-resident females ($F_{17,165}$=1.70, p=0.210).

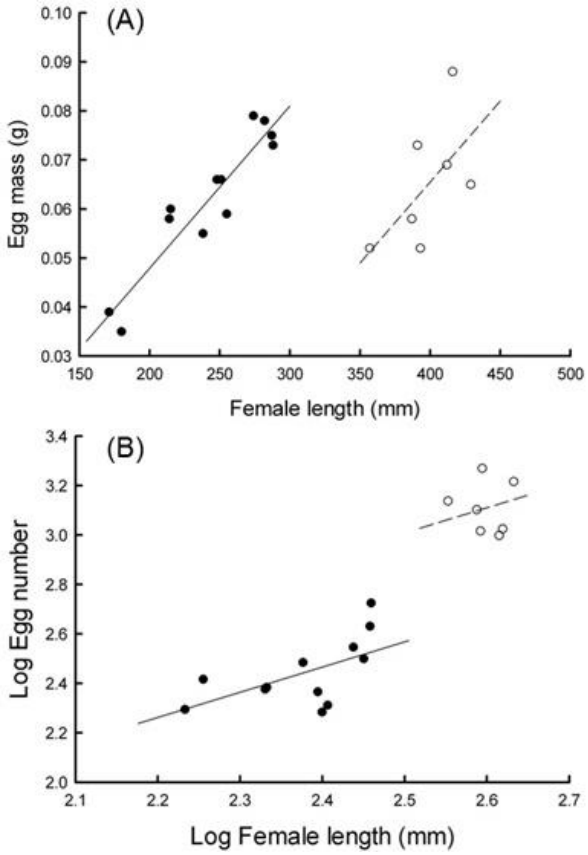

Fig. 2.2. The relationship between female length and egg mass (A) and egg number (B) for freshwater-resident (closed circles, solid line) and anadromous (open circles, dashed line) females. See text for statistical analysis.

2.3.3 Early Development

There was no difference in the survival rate of offspring of the two parental ecotypes from fertilisation to hatching ($F_{16,16}$=0.007, p=0.934). After controlling for the positive effect of egg mass on time to hatch ($F_{16,16}$=265.87, p=0.022; Fig. 2.3A), there was

a significant effect of parental ecotype, with eggs from freshwater-resident parents taking considerably longer to hatch than those from anadromous parents ($F_{16,16}$=56.92, p<0.001; Fig. 2.3A).

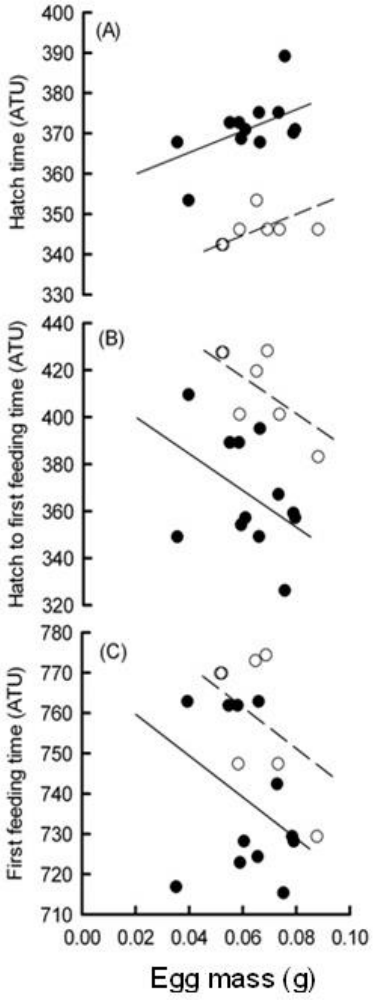

Fig. 2.3. The relationship between three development milestones (from fertilisation to hatching (A), from hatching to first feeding (B) and from fertilisation to first feeding (C)) and egg mass for freshwater-resident offspring (closed circles, solid line) and anadromous offspring (open circles, dashed line). Accumulated thermal units (ATU=days X average daily temperature). See text for statistical analysis.

However, while the mass of the alevin at hatching was positively related to egg mass (Fig. 2.4 A), it did not differ between parental ecotypes, regardless of whether egg size was taken into account (controlling for egg size: $F_{17,17}$=2.47, p=0.13; ignoring egg size: $F_{17,196}$=0.1582, p=0.70).

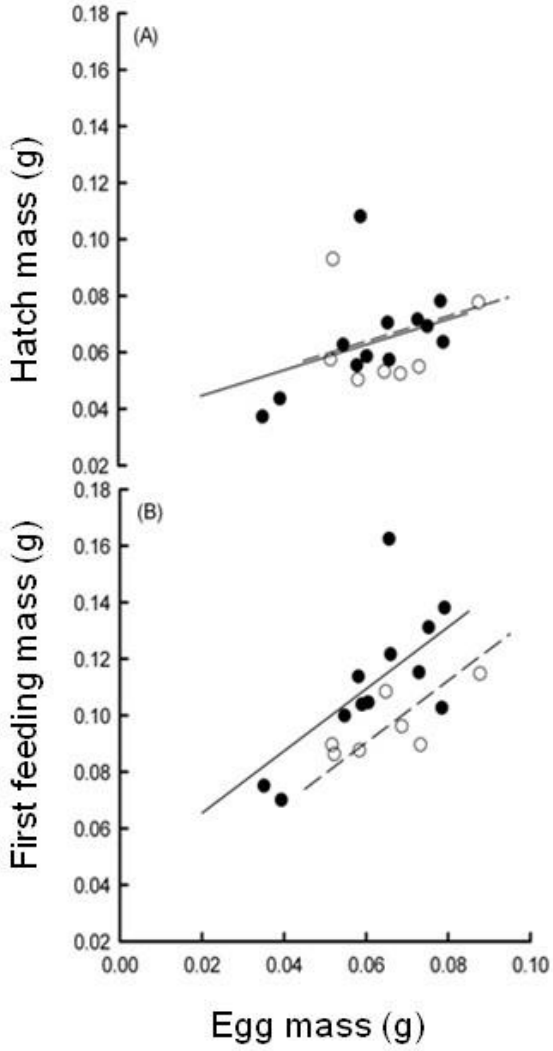

Fig. 2.4. The relationship between two developmental milestones (hatching (A) and first feeding (B)) and egg mass for freshwater-resident offspring (closed circles, solid line) and anadromous offspring (open circles, dashed line). See text for statistical analysis.

The mean mass (± SD) of freshwater-resident and anadromous offspring at hatch was 0.0648 ± 0.0517g and 0.0614 ± 0.0537 g respectively. There was no difference in the conversion efficiency of egg mass into hatching mass of the offspring from freshwater-resident and anadromous ecotypes ($F_{17,17}$=0.194, p=0.665; Fig. 2.5).

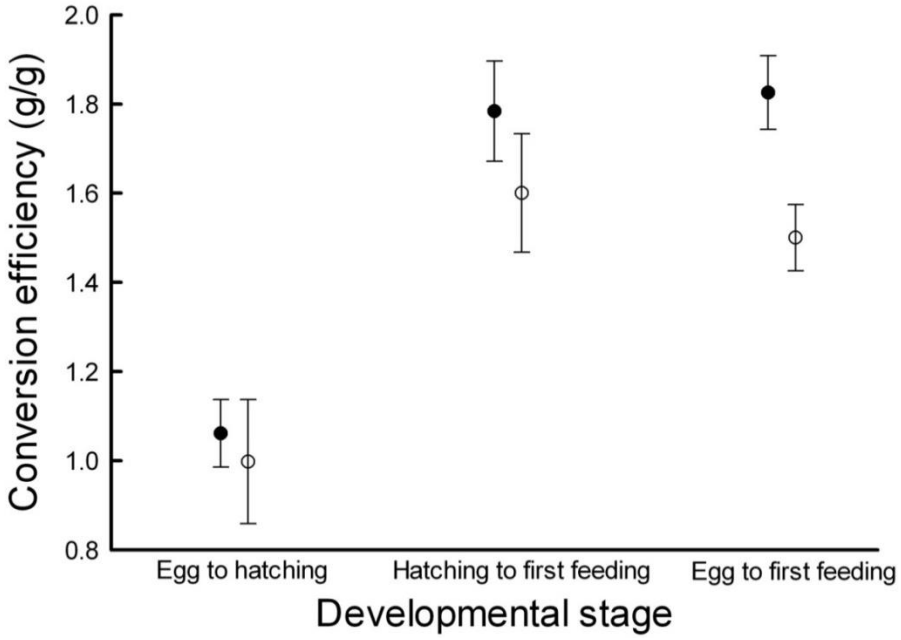

Fig. 2.5. The relationship between conversion efficiency at three developmental milestones (egg to hatching, hatching to first feeding and egg to first feeding for freshwater-resident offspring (closed circles) and anadromous offspring (open circles). Error bars represent standard error of the mean. See text for statistical analysis.

2.3.4 First Feeding

There was a significant difference between offspring of the two parental ecotypes in the later rate of development, with the time to first feeding of offspring of freshwater-residents being considerably shorter than that for offspring of anadromous fish, whether this is taken from the time of egg laying ($F_{17,17}$=5.48, p=0.032; Fig. 2.3C, pg.19) or from the time of hatching ($F_{16,16}$=4.97, p=0.041; Fig. 2.3B, pg.19). Offspring of freshwater-resident parents were significantly heavier at the time of first feeding than those of anadromous parents (mean mass $\pm$ SD 0.1080 $\pm$ 0.0245 g and 0.0903 $\pm$ 0.0152 g respectively, $F_{17,171}$=4.42, p=0.05). I found a positive correlation between egg mass and first feeding mass ($F_{16,16}$=15.03, p= 0.001; Fig. 2.4B, previous page); after controlling for this, offspring from freshwater-resident crosses were considerably larger than those from anadromous crosses, whether the analysis ignored ($F_{17,171}$=4.42, p= 0.05; Fig 2.4B,previous page) or controlled for maternal body length ($F_{15,171}$=15.12, p= 0.002). There was no difference in conversion efficiency between the hatch stage and first feeding stage ($F_{17,17}$=1.05, p=0.312; Fig. 2.5), but I did find a difference in conversion efficiency between the egg stage and first feeding stage, with offspring from freshwater-resident crosses converting egg mass to fry mass more efficiently ($F_{17,17}$=7.04, p=0.017; Fig. 2.5)

2.3.5 Metabolism

Mass was highly significant in all models for SMR, MMR and AS. There was no difference in SMR ($F_{16,54}$=0.034, p=0.858; Fig. 2.6A) , MMR ($F_{16,54}$=0.296, p=0.594; Fig. 2.6B) or AS ($F_{16,54}$=0.406, p=0.533; Fig. 2.6C) between freshwater-resident and anadromous offspring at the time of first feeding.

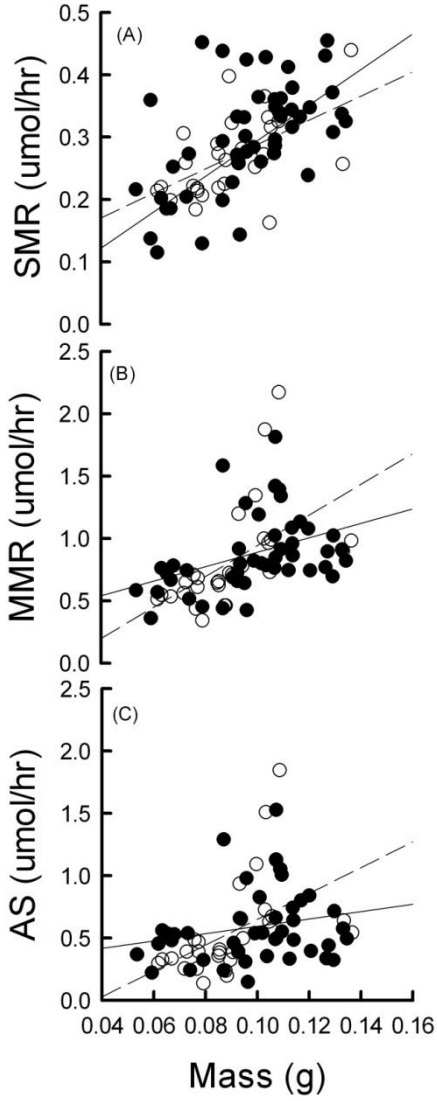

Fig. 2.6. The relationship between metabolic rate (SMR, MMR and AS) and mass for freshwater-resident offspring (closed circles, solid line) and anadromous offspring (open circles, dashed line). See text for statistical analysis but note that there were no significant differences between freshwater-resident and anadromous offspring in all metabolic measures.

2.4 Discussion

This study demonstrated that offspring from freshwater-resident and anadromous trout ecotypes differ significantly in their size and timing at various stages of early

development. Not surprisingly, given the lower productivity of temperate fresh waters relative to marine environments at the same latitude (Gross et al.1988), adult freshwater-resident trout were smaller than adult anadromous trout (Jonsson 1985; Jonsson & Jonsson 1997). Given their difference in body size, it was not surprising that anadromous trout produced more eggs than did freshwater-residents, but these eggs were smaller than those of freshwater-residents after controlling for maternal body length, a pattern consistent with sympatric brown trout populations found in parts of Sweden (Olofsson & Mosegaard 1999).

A reproductive strategy based on a greater number of smaller eggs (as was found for the anadromous females in this study) may be adaptive when habitat quality is high, as this may result in a greater return per reproductive effort (Brockelman 1975; Allen et al. 2008). As food availability in the environment increases, fitness amongst even the smallest individuals also increases, while the benefits of a larger egg (i.e. larger body size at hatch and/or greater energetic reserves) decreases, making production of more individuals, as opposed to fewer larger individuals adaptive (Rollinson & Hutchings 2013). The eggs of the freshwater-resident crosses hatched later than did those of anadromous crosses, but began feeding earlier and at a heavier mass. Since there was no difference in our study in relative energy density of the egg or mass at hatching between the offspring of the two ecotypes, this meant that freshwater-resident offspring had a higher efficiency of conversion from egg mass to fry mass, although differences in energy density of the eggs between ecotypes has been noted elsewhere (Jonsson & Jonsson 1997).

The negative relationship between egg size (corrected for maternal body length) and the time taken to complete the stage from hatching to the start of exogenous feeding has been supported in other taxa (although see Pakkasmaa & Jones 2002 and Rollison & Hutchings 2010 for exceptions). For example Dziminski et al. (2009) demonstrated that larger eggs of the quacking frog (*Crinia georgiana)* had a shorter larval period and were larger at metamorphosis. Large eggs generally give rise to large offspring at hatching (Reznick 1991; Roff 1992), which can confer benefits of greater competitive ability (Einum & Fleming 1999) and hence preferential access to food (Cutts et al.1999), although the incurred benefits of a larger body size at hatch may be species-dependent and may differ based on the degree of parental care and the dependence level of the offspring on exogenous food resources. However, given that salmonid fry must compete for preferred feeding territories when they have emerged from the gravel at the start of exogenous

feeding (Cutts et al. 1999; Van Leeuwen et al. 2011), a process in which prior residence is also important (Sloman & Armstrong 2002), a further advantage of producing larger eggs is that they had a reduced time taken to develop to the first feeding stage.

The relative time at which mothers spawn in the breeding season may also influence the viability of their offspring (Skoglund et al. 2012). Elliot & Hurley (1998) demonstrated that larger females within a population of anadromous brown trout spawned earlier in the season than did smaller members of the population. This did not appear to be the case in our study population, since the migratory females (which were larger than freshwater-residents) tended to be ripe later than the freshwater-residents, although these fish were held in the laboratory and were artificially stripped. Moreover, for a given egg size, the offspring from freshwater-resident crosses were larger at the onset of exogenous feeding than those from anadromous crosses, and reached that stage sooner. It would therefore be expected (assuming the observed patterns in our study are representative of those found in sympatric populations) that the offspring from freshwater-resident crosses would have a competitive advantage over anadromous offspring during early ontogeny. Given this initial size/prior residence advantage, the temporal stability of size based/dominance hierarchies in territorial juvenile salmonids (Bachman 1984; Abbott & Dill. 1985; Nakano 1995), and the likelihood of higher growth supporting a resident life history (Olsson & Greenberg 2004; Olsson et al. 2006), it seems likely that a higher proportion of offspring from freshwater-resident crosses would remain freshwater-resident whereas a higher proportion of offspring from anadromous crosses would undergo at least some form of migration in order to maximise their growth opportunity.

It is unclear what factors contributed to the differences in body size between the two offspring types at the start of exogenous feeding although it is likely that these arose from a difference in conversion efficiency between the egg stage and the start of exogenous feeding, with offspring from freshwater-resident parents converting a given egg mass into a greater fry mass. Morinville & Rasmussen (2003) demonstrated that individual migrant brook trout (*Salvelinus fontinalis)* had a higher food consumption rate and lower growth efficiency in the year prior to migration compared to sympatric resident brook trout. Therefore the pattern of higher conversion efficiency in offspring from freshwater-resident parents described at the embryo stage here may be maintained through ontogeny, although this remains to be tested in brown trout. Although not tested at the egg or alevin stage, one possible explanation for a difference in conversion efficiency would have been

differences in minimal (SMR) or maximal (MMR) metabolism although no subsequent differences in SMR, MMR or AS were observed at the first feeding stage in our study, indicating that at least during the early fry stages, differences in overall baseline energy expenditure and aerobic capacity are similar between offspring types.

Given the differences in development between the two types of offspring in our study we suggest that the migration history of the parents has the potential to influence the migration probability of the offspring. What remains unclear is whether the differences have a genetic basis (i.e. due to genetic differences between anadromous and freshwater residents), or are parental effects, possibly arising as a by-product of differing environments experienced by the two types of parents prior to spawning. There is a strong theoretical basis for variation in maternal provisioning of the egg arising as a by-product of differing environments. Mothers living in low food environments often exhibit reduced growth, smaller adult size and lower lipid reserves, and in turn tend to produce fewer but larger eggs (Taborsky 2006), whereas those in high food environments show greater growth, larger size and higher lipid reserves and tend to produce a greater number of smaller sized eggs (Burton et al. 2013). Furthermore Braun et al.(2013) demonstrated that in years when migration was challenged by high water discharges, female Sockeye salmon (*Oncorhynchus nerka)* invest less in gonadal development and produce smaller but not fewer eggs, consistent with other studies demonstrating a negative relationship between egg size and migration cost (Fleming & Gross 1990; Kinnison et al. 2001). Therefore, given both the likelihood of non-breeding partial migration to generate variation in the pre-breeding environment (Burton & Metcalfe 2014) and the further constraints due to migration, it follows that anadromous females should be producing a greater amount of smaller eggs compared to freshwater-resident females.

A limitation of this study is that the age and spawning history (maiden spawners versus repeat spawners) of the females was not known. Reid & Chaput (2012) found that repeat spawning females of the closely-related Atlantic salmon had a higher fecundity than maiden spawning females but produced smaller eggs with lower survival. Furthermore, in other species it has also been shown that younger females for a given body size tend to produce smaller eggs (Quinn et al. 2011; Burton et al. 2013). Given the lower probability of repeat spawning in anadromous fish (due to higher mortality associated with migration) compared to freshwater-resident fish, it is possible that age and spawning history of the females could have influenced the patterns that we observed. A second limitation is that I

was unable to determine whether the differences between offspring were primarily due to genetic or maternal effects. This would be difficult to establish given that the resident-anadromous dichotomy by its very nature prevents the use of the standard approach of rearing the parents in a common garden to rule out maternal effects. It is possible that the ontogenetic patterns described here may be reduced or accentuated in the wild. Freshwater-resident and anadromous females may use different spawning habitats and have different spawning behaviour. For example larger fish (i.e. anadromous fish in our study) have been shown to dig deeper nests and utilise larger substrates which could potentially influence developmental characteristics which may not necessarily be representative under benign laboratory conditions.

In conclusion, the results of this study demonstrate that offspring from freshwater-resident and anadromous life history strategies differ considerably in early development but in a way that is consistent with literature-based predictions for triggers of migration (i.e. migratory fish have smaller eggs for a given maternal body size, while fry from larger freshwater-resident eggs begin feeding sooner and at a larger size). Therefore we suggest that parental effects (either direct or environmentally-mediated through differences in rearing environments) may be an important mechanism underlying the expression of threshold traits and play a significant role in the perpetuation of non-breeding partial migration within populations.

Chapter Three: The Association between Parental Rearing Environment and Offspring Performance

*Note: A version of this chapter has been accepted as a manuscript to the Journal of Experimental Biology.

3.1 Introduction

In many taxa there is considerable intraspecific variation in life-history strategies within a single population (Skov et al. 2008; Chapman et al. 2011; Mehner & Kasprzak, 2011), reflecting alternative routes through which organisms can achieve successful reproduction (Roff 2002; Eberhard 2003). Intraspecific differences in life-history strategies can be manifested in terms of variation in age or size at reproduction, level of reproductive investment per breeding attempt or per propagule, or strategy used to obtain a mate (e.g. fighting vs. sneaking males). While some of the variation in life-history strategies can likely be explained by differing environments experienced by the individual during its development (Monaghan 2008), there is a growing body of literature suggesting that a considerable amount of the variation could be attributed to parental effects, whereby the conditions experienced by the parents during their own life can leave a lasting legacy on the generations to follow (Burton & Metcalfe 2014). These non-genetic influences are sometimes termed maternal effects, but we use the term 'parental' since the father can also have an influence.

Much of the work investigating parental effects has focused on understanding how the direct effect of the condition and/or current environment of the parents at the time of reproduction shapes propagule size (Mousseau & Fox, 1998), number (Einum & Fleming, 2000) and quality (Blount et al. 2002; Burton et al. 2013). While it is undeniable that these parental effects can have dramatic fitness consequences for the developing offspring, their importance is unclear (Uller et al. 2013) and it remains debated as to the adaptive nature of these effects since this would presume that juvenile and adult ecologies are correlated (i.e.: the environment experienced by the parents around the time of spawning is the same for the developing offspring) which is not likely in species that adopt alternative and/or complex life histories (Taborsky 2006). More likely is that the juvenile rearing environment and the life history strategy of the parents may be a better predictor for the performance and life history pathway of their offspring (Taborsky 2006; Jonsson & Jonsson 2014).

Across taxa, Atlantic salmon show some of the greatest within-population variability in life history strategies (Fleming 1996; Garant et al. 2002). In this species, multiple discrete male and female life histories co-exist and interbreed on many spawning grounds (Thorpe & Metcalfe 1998), although the effect of the various combinations of life histories on offspring traits remains unknown. Males and females can spend between one and six years in fresh water prior to smolting (the physiological and morphological preparation for salmonids to enter sea water) and emigrating to sea, where they spend one or more winters at sea before returning to their natal freshwater stream to spawn. The range in time spent in fresh water prior to seaward migration is primarily due to variation among rivers in growth conditions, with fish migrating at a younger age in warmer rivers (Metcalfe & Thorpe 1990), but there is also variation in age at migration within rivers. Interestingly, however, some males, generally those that exhibit fast early growth (Whalen & Parrish 1999; Aubin-Horth & Dodson 2004), will become sexually mature at a small size without ever going to sea; these males, known as precocious male parr, participate in spawning as sneakers (Fleming 1996).

Since fast growing juveniles tend to migrate to sea earlier than their slower growing counterparts from the same population (Metcalfe 1998), there has been interest in the intrinsic differences between fish that affect potential growth rate and hence generate life history variation. One trait which has received a considerable amount of attention is metabolic rate (Forseth et al. 1999), partly because it is often correlated with dominance or growth (Metcalfe et al. 1995; Álvarez & Nicieza 2005) and partly because it constitutes the fundamental energy budget of organisms. Standard metabolic rate is the minimal maintenance metabolic rate of an ectotherm in a post-absorptive and inactive state (so is the equivalent of the basal metabolic rate of an endotherm). Standard metabolic rate (usually measured in terms of oxygen consumption) is an integrated measure of the energy expenditure involved in tissue maintenance and organism homeostasis. After controlling for temperature, body size and other sources of variation, SMR often differs by a factor of 2 or 3 between individuals of the same age, sex and species held in similar conditions (Burton et al. 2011). Aerobic scope is defined as the difference between an animal's SMR and its maximum possible aerobic metabolic rate (MMR) under the same environmental conditions, so that aerobic AS defines the capacity of the animal to increase its rate of aerobic metabolism (Killen et al. 2012).

Individual differences in SMR within salmon and trout populations have been linked to variation in individual growth and life history strategies (e.g. timing of smolt migration; Metcalfe et al. 1995; Forseth et al. 1999; Finstad et al. 2007). In contrast, the role of AS remains relatively understudied, although it has been found to be correlated with swim performance, distance of migration (Eliason et al. 2011) and survival of individuals in challenging environments (Clark et al. 2011; Killen et al. 2012). However, the associations between these traits and how they may influence the life history of their offspring remains unknown. Here we use the diversity of life history strategies in Atlantic salmon and the scope they offer for controlled *in vitro* fertilisations to test (1) whether there is any association between maternal or paternal life history pathway and offspring performance, (2) whether the strength of any such effects weakens over time during offspring development, and (3) whether parents that exhibited fast juvenile growth produce offspring that themselves have higher growth and metabolic rates.

3.2 Methods

3.2.1 Broodstock Collection and Crosses

In November 2012 mature sea-run Atlantic salmon undertaking their spawning migration were captured at the Loch na Croic fish trap on the river Blackwater, northern Scotland (57° 60'N, 4°63'W). Males and females were held separately at the trap site until ripe in circular tanks (4.0m diameter, 1.5m deep) supplied directly with water from the river Blackwater. Precocious male parr were captured by electrofishing on the river Blackwater each day that they were required for spawning trials (see below). The life history of each fish was determined using scalimetry (whereby the pattern of circuli of a fishes scale is examined and the various stages of ontogenetic growth revealed). Results of the scalimetry analysis revealed that all females were maiden spawners and had spent either two or three years in fresh water and one (1SW) or two years (2SW) in salt water. Males had spent one, two or three years in fresh water and zero, one (1SW) or two years (2SW) in salt water prior to spawning; the males that had spent zero years in salt water were precocious male parr that would adopt a sneaking spawning strategy (Fleming 1996). Average lengths were 578.6 $\pm$ 20.9mm and 728.9 $\pm$ 46.2mm for 1SW and 2SW females, and 117.5 $\pm$ 11.3 mm, 578.1 $\pm$ 47.04mm and 792.9 $\pm$ 45.6 mm (mean $\pm$ SD) for precocious parr, 1SW and 2SW males.

In order to evaluate the effect of maternal and paternal fresh water and salt water rearing environment on offspring traits we used a factorial mating design (Fig. 3.1).

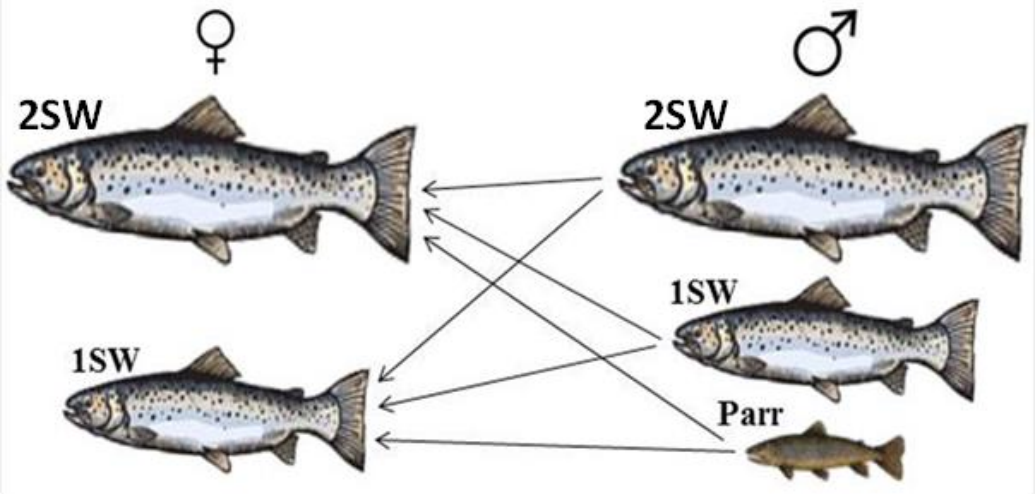

Fig. 3.1. Schematic diagram of the factorial mating design adopted for the study. See text for more details.

Eggs or sperm were extruded by abdominal massage from anaesthetised ripe fish. Eggs from each female were collected, drained of ovarian fluid and weighed to the nearest 0.1g on the day of stripping to determine clutch mass. A 1SW and a 2SW female (assigned to sea ages on the basis of size, later confirmed by scalimetry) were paired, and their clutches each split into three and fertilised with sperm from a single haphazardly chosen male of each life history type (precocious parr, 1SW and 2SW). Each mating block of five fish (2 female types x 3 male types) was then replicated nine times using different individuals (giving 54 families, derived from a total of 45 parents, 9 of each parental type). All parental fish were blotted dry and measured (fork length $\pm$0.5cm; body mass $\pm$0.1 g) after collection of their gametes. Four families had high infertility or embryo mortality during early incubation, leaving 50 families available for experimentation. All fish spawned from 28-30 November, 2012.

3.2.2 Egg Rearing, Hatching and Experimental Procedures

Fertilised eggs were transferred from the capture site to the nearby SSE hatchery at Contin, Scotland, where they were reared as separate family groups, under ambient water temperatures until the eyed stage. Dead eggs were recorded and carefully removed daily.

On 5 March 2013, 50-100 eyed eggs from each family were transferred to the aquarium facilities on the campus of the University of Glasgow, Scotland, where they were maintained as separate family groups in sections (one per family) of an experimental stream tank which was transversely divided using 1mm nylon mesh. Incubation temperature during this period was slowly raised from 5 to 13°C over the course of 30 days

to increase developmental rate. Eggs hatched from 25 March - 3 April 2013. On 22 April 2013, by which time they had reached the first feeding stage (the point in time when the alevins' yolk sac is fully exhausted and individuals begin to swim up from the substrate in search of food), 10 fry per family were anaesthetised, blotted dry and measured (fork length ±0.1mm; body mass ±0.0001 g). At this stage fish continued to be held in the same experimental stream tank described above on a recirculation system, but whereas previously they had been held in darkness they were now kept under a simulated ambient photoperiod. Fish were fed *ad libitum*, several times daily, on a standard commercial salmon pellet (Biomar, Aarhus, Denmark) recommended for the particular life stage of fish.

On 30 July 2013 10-15 fry from each of the remaining 50 families were anaesthetised, measured (fork length ±0.1mm; body mass ±0.0001 g), tagged with a visible implant elastomer (Northwest Marine Technology, Inc.) and transported to the Scottish Centre for Ecology and the Natural Environment, Scotland. One individual from each of the 50 families was stocked into one of ten, 15 L (50cm X 30cm X 15cm) clear plastic aquaria. The aquaria were placed inside a constant temperature room on a partial recirculation system at a temperature of 13.6 ± 1 °C (mean±SD), with a simulated ambient photoperiod. Fish were fed approximately 3 % body wt/ day on a standard commercial salmon pellet (Biomar, Aarhus, Denmark) for the remainder of the experiment. Fish were given a two week period to acclimate to the new rearing environment before being anaesthetised and re-measured on 12 August 2013. They were then anaesthetised and re-measured on 13 September 2013, so that their growth rate over the preceding 32 day period could be calculated. All fish were then subjected to metabolic measurements.

3.2.3 Measuring Standard Metabolic Rate

Aquaria were vacuum siphoned to remove food and debris the day before fish were placed in respirometry chambers. This ensured that fish were unfed for at least 28 h prior to oxygen uptake measurements, and had sufficient time to evacuate their guts; 28 h post-feeding has been shown to be adequate for the specific dynamic action (SDA) response to subside in salmonids (Cutts et al. 2002). SDA is an elevation in metabolic rate from the increased energy demands associated with digestion, immediately following a meal (Rosenfeld et al., 2015), and is generally not considered part of SMR.

Oxygen uptake was measured continuously over a 9-11 h period, from approximately 14.00h onwards, using flow-through respirometry (Van Leeuwen et al.

2011; 2012). Individual fish were placed into one of 24 separate glass respirometry chambers (either 8.0 cm length, 3.4cm diameter or 15 cm length, 3.4cm diameter, depending on fish size). Chambers were submersed in a water bath housed inside a second constant temperature room kept at the same temperature (13.6 $\pm$ 0.5 °C across all measurements) as the tanks in which growth was measured. An air-stone in the header tank of the respirometer apparatus kept the inflow water fully saturated with oxygen. Chambers were wrapped in dark plastic to prevent visual contact between individual fish during measurements, and all measurements were conducted in the dark to further minimise fish activity (Cutts et al. 2002). Glass respirometers and tygon tubing were used to minimise potential issues with use of plastics and oxygen permeable materials (Stevens 1992). Flow to each respirometer was adjusted using a nylon bodied micro valve (The West Group, Hampshire, UK) to ensure that the oxygen concentration dropped by 10 – 20% between the inlet and outlet of the respirometer chamber. Flow was measured by collecting the outlet water for a period of 30 s and weighing to the nearest 0.0001g.

Oxygen concentration of the outflow water was measured using one of three oxygen meters (FireSting O_2 oxygen meter; PyroScience GmbH) each fitted with four oxygen probes (calibrated daily) which were placed in small collection tubes connected to the outlet side of each respirometer chamber. Oxygen probes were rotated from the first 12 respirometers to the remaining 12 respirometers after approximately 9-11 hours of continuous measurement, allowing for 24 fish to be measured daily.
The rate of oxygen consumption was determined using the following equation (Ege & Krogh 1914):

$$MO_2=V_w(\Delta C_w0_2)$$

Where V_w is the flow rate of water through the respirometer and ΔC_w0_2 is the difference in oxygen tension between water entering and leaving the respirometer. Oxygen concentration was calculated by correcting PO_2 (partial pressure of oxygen) for barometric pressure and multiplying by $\alpha O2$ (μmol L^{-1} $torr^{-1}$), the solubility coefficient at the observed temperature (Van Leeuwen et al. 2011). Rates of background oxygen consumption were then subtracted from the observed values by measuring the oxygen concentration of the outflow water in the absence of fish at the beginning and end of each measurement trial and assuming a linear decrease in oxygen concentration over the measurement period.

Measurements of oxygen uptake were plotted graphically allowing for periods of complete rest to be readily discriminated from spontaneous activity, which appeared as

distinct spikes. Standard metabolic rate was estimated by using the lowest 10 min running average of oxygen consumption observed during the respirometry trial.

3.2.4 Measuring Maximal Metabolic Rate

After SMR had been measured individual fish were sequentially subjected to an exhaustive chase protocol in order to determine their MMR (Reidy et al. 1995; Killen et al. 2010; Norin 2014). A single fish was introduced into a round circular arena and hand-chased for three minutes to exhaustion. The fish was then immediately placed into one of two glass respirometry chambers as described above. Chambers were submersed in a water bath housed inside the same constant temperature room as the apparatus used for measuring SMR. An air-stone in the water bath of the respirometer apparatus kept the water fully saturated with oxygen. Oxygen uptake was measured using intermittent flow-through respirometry (Steffensen 1989). Once a fish was placed in the respirometer chamber the flush pump (Eheim 300 universal, Deizisau, Germany), which delivered oxygenated water through the respirometer, was immediately turned "off" which allowed for the rate of oxygen depletion (due to fish respiration) to be measured. During this "off" phase a peristaltic pump (Masterflex L/S, London, England) was used to ensure adequate mixing within each of the two respirometers. Water oxygen concentration was measured every second until oxygen saturation levels reached approximately 85%. At this point the flush pump was switched "on" until oxygen saturation levels had restored and the cycle was repeated for a second time. Oxygen concentration within the respirometer was measured using the same oxygen meter (FireSting O_2 oxygen meter; PyroScience GmbH) fitted with the same oxygen probes as above. The rate of oxygen consumption was determined using the following equation (Ege and Krogh 1914):

$$MO_2=V_w(\Delta C_w0_2)/\Delta t$$

where V_w is the volume of water in the respirometer and associated tubing minus the volume of the fish and ΔC_w0_2 is the change in oxygen tension of the water over time period Δt (Steffensen 1989). Oxygen concentration was calculated by correcting PO_2 (partial pressure of oxygen) for barometric pressure and multiplying by αO2 (μmol L^{-1} $torr^{-1}$), as before. Maximal metabolic rate was determined as the higher oxygen consumption rate of the two measurements. In most cases this value corresponded to the first measurement immediately following the exhaustive chase protocol. Following respirometry measures all fish were anaesthetised, and weighed to the nearest 0.0001g.

3.2.5 Calculations and Statistical Analyses

Offspring condition was calculated as relative condition factor, K_{rel}, according to Froese (2006) using the following equation:

$$K_{rel} = W/aL^b$$

where W is the mass of the individual offspring in grams, L is the fork length in centimetres and a and b are the exponential form of the intercept and slope derived from the regression of mass vs. length plotted on double logarithmic axes for all the offspring combined. Specific growth rates of fish (percent per day) were calculated as $100[\log_e(\text{final mass}) - \log_e(\text{initial mass})]/\text{duration}$ (Ricker 1975). Aerobic scope was determined by subtracting MMR from SMR. Given the large variation in fish size and the confounding effect of size on metabolism we used residual SMR, MMR and AS in subsequent analysis. These residual values were calculated as residuals from the regression of absolute oxygen consumption (SMR, MMR or AS, umol/hr) vs. mass (g) for the full sample size of fish, plotted on double logarithmic axes.

We used linear mixed effects models (LME) to test for the effects of maternal and paternal time spent in fresh water and salt water on offspring traits (length, mass, condition, growth, SMR, MMR and AS). All LME models initially included all possible two way interactions, with maternal and paternal identities included as random factors to control for the non-independence of siblings. Variance inflation factors (VIF's) for all explanatory variables were calculated prior to analysis; all VIF's were less than 3, indicating that collinearity among explanatory variables was unlikely to have affected the analyses (Zuur et al. 2009). Furthermore, visual inspection of residual plots did not reveal any obvious deviations from homoscedasticity or normality. Likelihood ratio tests comparing models with and without a given term were used to sequentially compare model fit; models were progressively simplified provided that any increase in the log-likelihood ratio statistic was non-significant ($p > 0.05$). For those instances where a single term could not be isolated from the model, due to a significant interaction present, we compared the single factor best-fit model with and without the single term in question. All analyses were conducted using R version 3.0.1 statistical software (R Core Team, 2013) and the lme4 function (Bates et al. 2012).

3.3 Results

3.3.1 First Feeding Offspring

There was no effect of the amount of time that parents had spent in fresh water as juveniles on offspring length, mass or condition at the time of first feeding. However we did find a positive relationship between the amount of time mothers had spent in salt water prior to spawning and offspring mass (χ^2=15.0, df= 1, p<0.001; Fig. 3.2A), length (χ^2=7.08, df= 1, p=0.008; Fig. 3.2B) and condition (χ^2=9.54, df= 1, p=0.002; Fig. 3.2C).

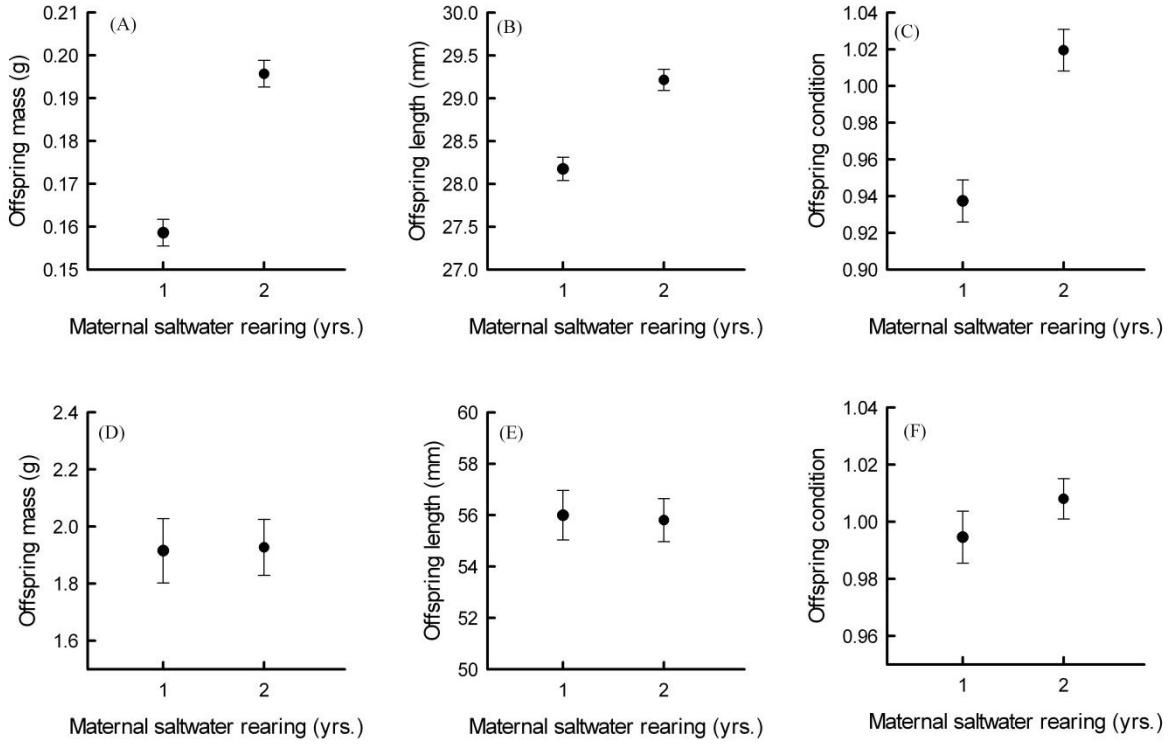

Fig. 3.2. The relationship between the time mothers had spent at sea (1 or 2 years) and three fundamental early fitness attributes (offspring length, offspring mass and offspring condition) for first feeding fry (A,B,C) and four month old fry (D,E,F). Note the loss of statistical significance by four months of age. Error bars represent 95% confidence intervals. See text for statistical analysis.

There was also a significant effect of the amount of time fathers had spent at sea on the condition of their offspring (χ^2=7.88, df= 2, p=0.02; Fig. 3.3, next page), with those fathered by precocious parr having a higher condition factor (Fig. 3.3, next page). Lastly we also found a significant interaction between the amount of time mothers had spent at sea and the amount of time fathers had spent in freshwater on offspring length (χ^2=9.48, df= 2, p=0.009) and condition (χ^2=20.32, df= 2, p=0.018).

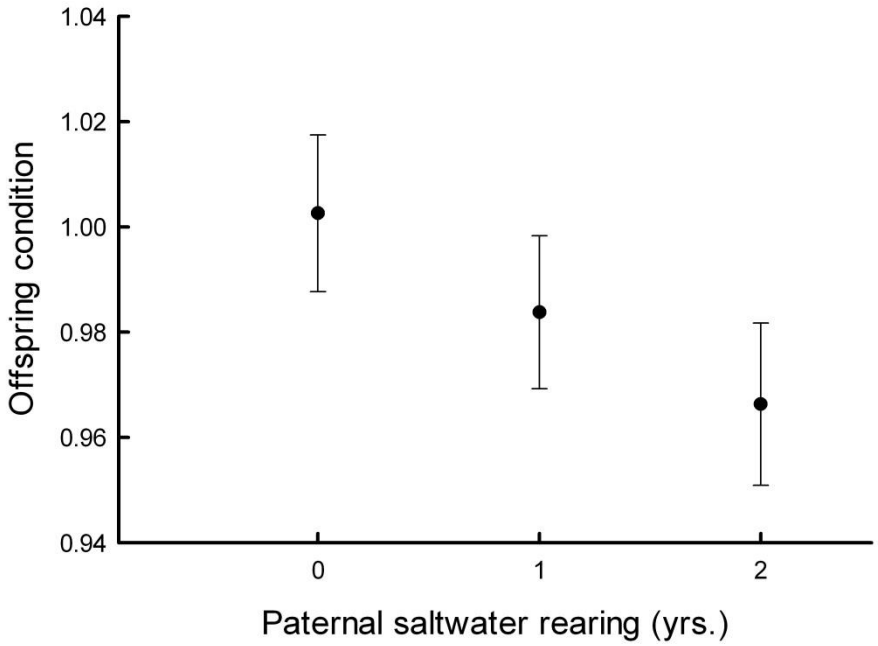

Fig.3.3. The relationship between the time fathers had spent in saltwater (0, 1 year and 2 years) and the body condition of their offspring at first feeding. Error bars represent 95% confidence intervals. See text for statistical analysis.

3.3.2 Post Feeding Offspring

The effects of the amount of time mothers had spent in salt water on offspring mass, length and condition had disappeared by the time that the fry were 4 months old (Fig. 3.2 D-F, previous page). Instead, it was the maternal duration in fresh water that was significant, being negatively related to offspring condition (χ^2=5.86, df= 1, p=0.02; Fig. 3.4A, next page), growth rate (χ^2=4.28, df= 1, p=0.04; Fig. 3.4B, next page), MMR (χ^2=5.15, df= 1, p=0.02; Fig. 3.4C, next page) and AS (χ^2=4.37, df= 1, p=0.037; Fig. 3.4D, next page); mothers that were faster to develop in fresh water (migrating to sea at two rather than three years of age) produced offspring that were in better condition, had higher rates of MMR, AS and grew faster, although there was no effect on SMR.

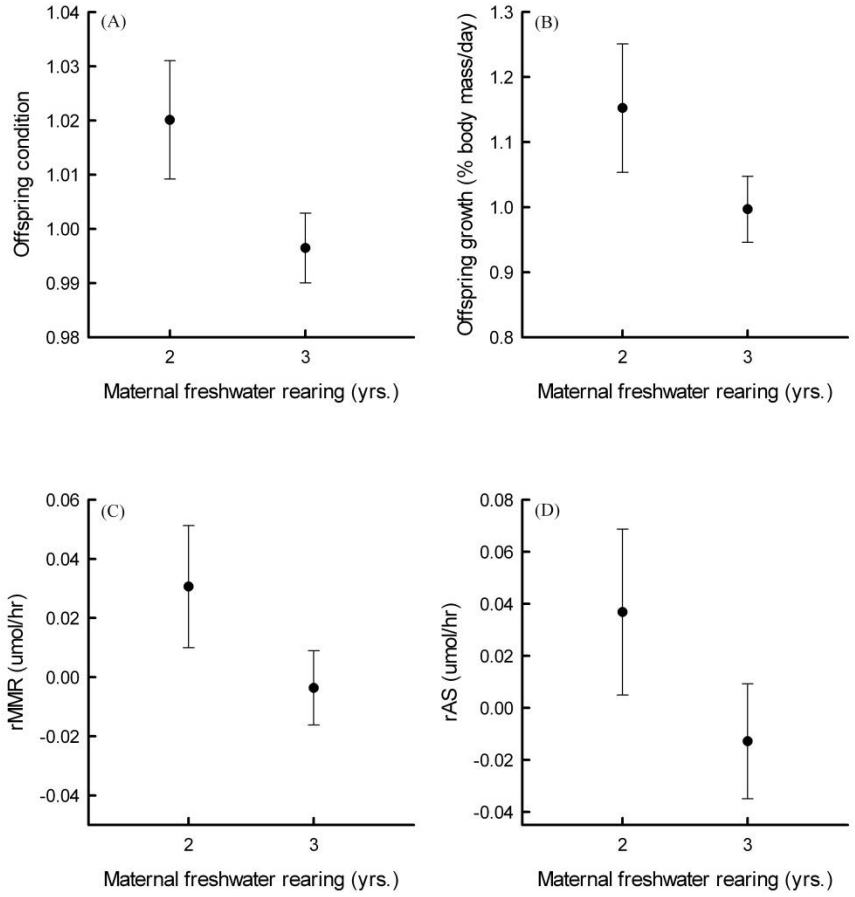

Fig. 3.4. The relationship between the time mothers had spent in freshwater (2 and 3 years) and the body condition of their offspring at 4 months of age (A), growth (B), MMR (C) and AS (D). Measures of metabolic rate are expressed as residuals, after correction for body mass (rMMR; rAS). Error bars represent 95% confidence intervals. See text for statistical analysis.

A significant interaction was found between the amount of time mothers had spent in salt water and the amount of time fathers had spent in fresh water on offspring growth (χ^2=6.78, df= 2, p=0.034), with offspring of the fastest developing parental life history types (i.e. mothers that had spent 1 year at sea and precocious parr fathers) having the fastest growth (Fig. 3.5, next page).

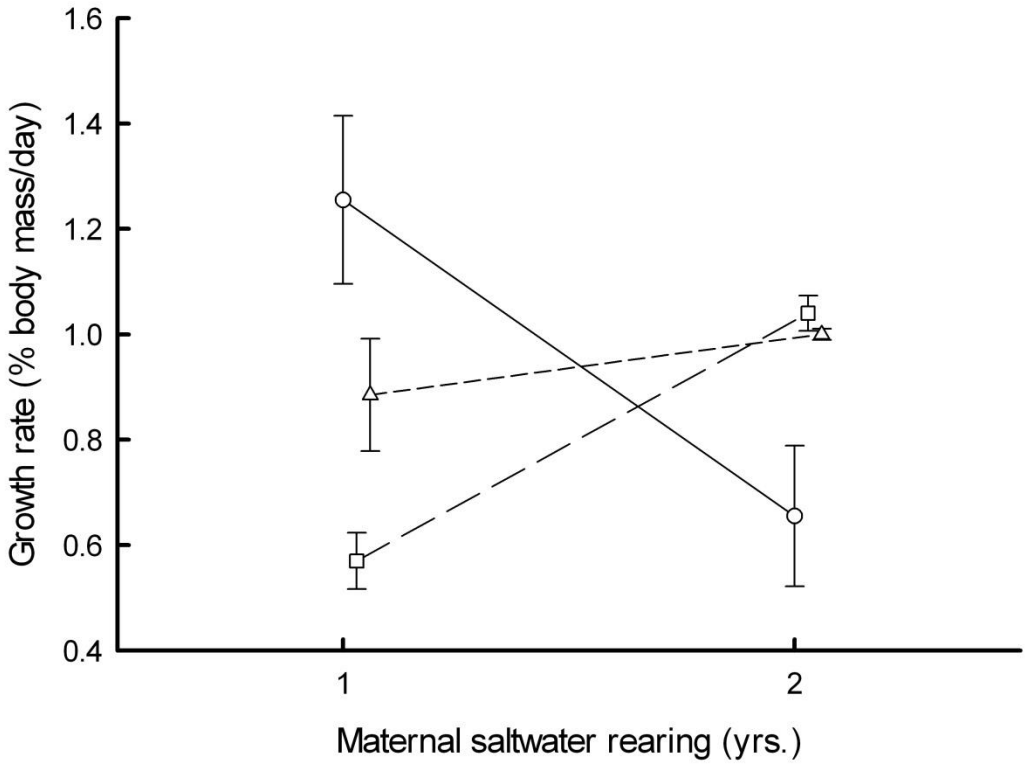

Fig. 3.5. The effect of the time mothers had spent in salt water (1 or 2 years) and fathers had spent in fresh water [1 year (open circles, solid line), 2 years (open squares, long dashed line) and 3 years (open triangles, short dashed line)] on the growth rate of their fry at 4 months of age. Error bars represent 95% confidence intervals. See text for statistical analysis.

Furthermore there was a significant interaction between the amount of time the two parents had spent at sea on offspring SMR (χ^2=8.93, df= 2, p=0.01), with offspring from parents that each had spent the longest period at sea (2 years) having the highest SMR (Fig. 3.6, next page).

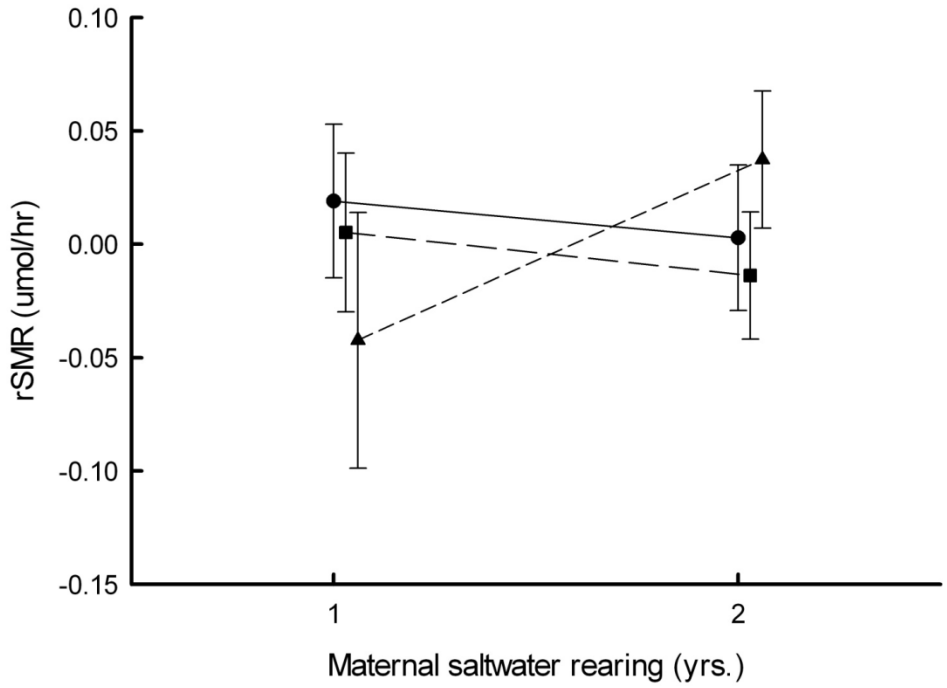

Fig. 3.6. The effect of the time mothers had spent in salt water (1 or 2 years) and fathers had spent in salt water [0 year (closed circles, solid line), 1 year (closed squares, long dashed line) and 2 years (closed triangles, short dashed line)] on the standard metabolic rate of their fry at 4 months of age. Measures of metabolic rate are expressed as residuals, after correction for body mass (rSMR). Error bars represent 95% confidence intervals. See text for statistical analysis.

Lastly, there was a significant interaction between the amount of time the two parents had spent in fresh water on offspring MMR (χ^2=7.45, df= 2, p=0.02), with offspring from the fastest developing parents in fresh water having the highest MMR (Fig. 3.7, next page).

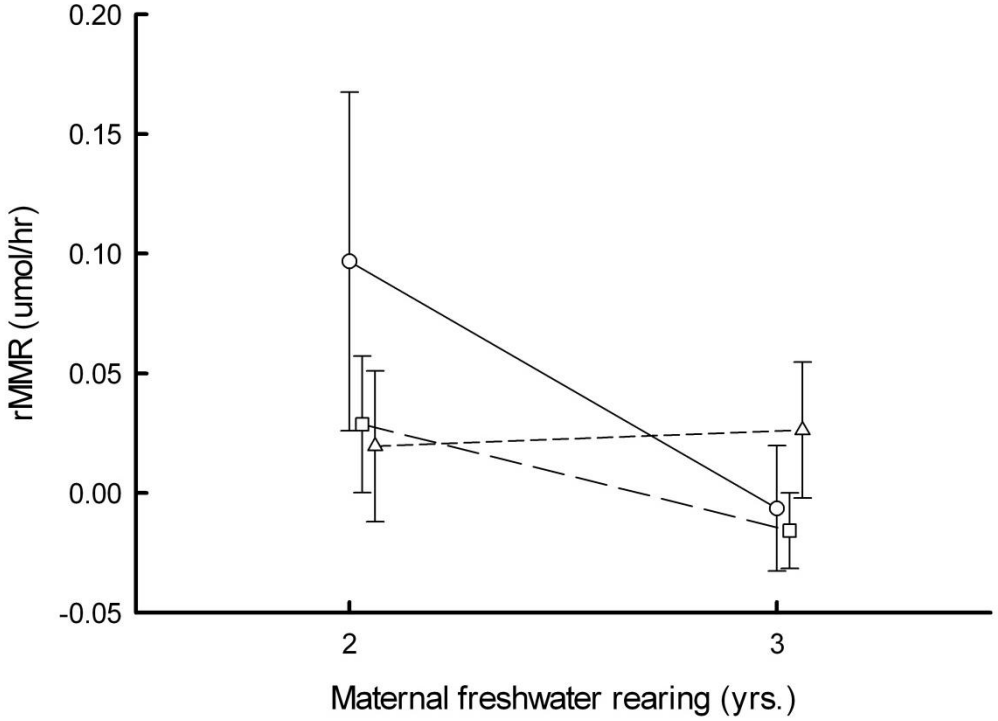

Fig. 3.7. The effect of the time mothers had spent in fresh water (2 and 3 years) and fathers had spent in fresh water [1 year (open circles, solid line), 2 years (open squares, long dashed line) and 3 years (open triangles, short dashed line)] on the maximum metabolic rate of their offspring at 4 months of age. Measures of metabolic rate are expressed as residuals, after correction for body mass (rMMR). Error bars represent 95% confidence intervals. See text for statistical analysis.

3.4 Discussion

This study demonstrated that the life history strategy of the mother, and to a lesser extent the father, was significantly associated with a range of offspring traits. The mother's life history was found to be significant throughout early offspring development, with the duration of time spent by the mother in both fresh water and salt water being associated with offspring traits related to early growth and metabolism. Mothers that had spent longer at sea (two versus one year) produced offspring which were heavier, longer and in better condition at the time of first feeding; this suggests that the effect is mediated through the provisioning of the egg since up until this point in development the embryo has been dependent on egg reserves. Similar effects are found in other taxa, for example the migratory duration and condition of female snow geese (*Chen caerulescens atlantica*) can influence the clutch size of eggs (Bety et al. 2003). Interestingly, early development in the salmon in the present study was not only due to maternal provisioning, since there was also a paternal effect at this stage, with offspring fathered by precocious parr having a higher body condition at the time of first feeding. These relationships had disappeared by the time

that the fry had been feeding on exogenous food for four months. At this stage, the juvenile rearing environment (time spent in fresh water) of the mother was a better predictor of offspring traits, with mothers that were faster to develop in fresh water (migrating to sea at two rather than three years of age) producing offspring that had higher MMR, AS and that grew faster.

Much of the work investigating maternal effects has focused on understanding how a mother's state around the time of reproduction affects the phenotype of her offspring (Bernardo 1996). There are a number of ways that mothers providing no parental care can nonetheless affect offspring traits through non-genetic means. One route is through the level of reproductive investment, in terms of either the overall amount of nutrients, or specific modifiers such as maternal hormones, that are deposited in the eggs. Rossignol et al. (2010) and Régnier (2012) found that the routine metabolic rate of Atlantic salmon fry and the SMR of brown trout embryos respectively decreased with increasing egg size (an indicator of general nutrient investment), while Sloman (2010) showed that SMR in brown trout fry could be affected by maternal cortisol deposited in the egg. Even the position of the egg within the ovary can influence metabolic and growth traits in the resulting offspring (Burton et al. 2013a).

While it is undeniable that these processes, presumably arising as a result of the female's physiological state at the time of reproduction, can have dramatic fitness consequences for the developing offspring, there is a growing body of literature that suggests that non-genetic influences on offspring phenotype may also arise as a consequence of the conditions that parents of both sexes experienced in early life (Taborsky 2006; Burton et al. 2013b; Burton & Metcalfe 2014). In this study we found that offspring traits were related to the early as well as the pre-reproductive period of the parents. Mothers that had spent a greater time at sea (two years at sea versus one year) immediately prior to reproduction produced offspring which were heavier, longer and in better condition. Because mothers that spend longer at sea are generally larger (likely due to the higher productivity in marine environments compared to adjacent freshwater environments (Gross et al. 1988)), this result was not surprising given that larger female Atlantic salmon tend to produce larger offspring (Burton et al. 2013b). These relationships between maternal marine life history and offspring traits had disappeared by the time the fry were four months old, a finding consistent with Donelson et al. (2009) who found that parental effects on juvenile size in the spiny chromis damsel fish (*Acanthochromis*

polyacanthus) were evident up to 29 days post hatching, but had disappeared by 50 days. However, we found that the growth, MMR and AS of the 4 month old fry in our study were in turn predicted by the mother's earlier life history (i.e. time spent in fresh water), with mothers that were faster to develop in fresh water producing fry that were faster growing and had higher maximal rates of metabolism.

Given that age of smolting and seaward migration in salmonids is principally determined by growth rate, whereby fast growing juveniles tend to migrate to sea at a younger age than slower growing counterparts (Metcalfe & Thorpe 1990), and given the role of metabolism in shaping growth potential, our data suggest that offspring of mothers that spent the minimal period in fresh water may themselves be more likely to migrate at a young age, provided that they experience an environment favourable for growth. While it is impossible to exclude the possibility that these are additive genetic effects, there is experimental evidence of a significant non-genetic component to migration age in salmon (Metcalfe 1998) and so mothers may be producing offspring that have traits that suit the environment the mothers themselves experienced when young, so that they are tailoring the life history trajectory of their offspring (Jonsson & Jonsson 2014).

While offspring traits were found to be more related to maternal than paternal life histories, there were still some significant associations with the father's reproductive strategy. Males of many species display alternative mating tactics (Brockmann 2001, Dodson et al. 2013). Two of the most common tactics include the territorial tactic (which would naturally be adopted by 1SW and 2SW male Atlantic salmon), whereby a male defends and monopolises a single female against other males, and the non-territorial/sneaker tactic (adopted by precocious parr) whereby a male (generally considerably smaller in size) steals fertilisations away from the territorial male by darting in and out of the nest area at the time of egg release (Garant et al. 2002; Neff 2004).

While both male reproductive tactics can be successful there remains a paucity of studies examining the relationship between a male's reproductive tactic and the viability and performance of his offspring. However, there is some evidence that females may derive considerable benefits from mating with non-territorial males, despite their apparently lower social status. For example, studies done on Atlantic salmon (Garant et al. 2002) and Bluegill sunfish (*Lepomis macrochirus*; Neff 2004) found significantly higher growth rates and swim performance in offspring fathered by sneaker males, consistent with

the higher condition, growth and MMR of offspring fathered by precocious parr males in our study (although the higher growth and MMR found in our study also depended on the life history of the female). However, whether mating with a non-territorial or sneaker male results in higher fitness overall for the mother is still unclear, since there are many components that will determine the viability and reproductive success of her offspring (similar uncertainty over their adaptive value surrounds the analogous situation of extra-pair offspring in species that form pair bonds, despite the intensity of research on that topic (Forstmeier et al. 2014; Hsu et al. 2014 and references therein). Moreover, the mechanism through which a male's reproductive tactic is associated with offspring traits and performance is unclear, since it could be through genetic or epigenetic means.

In conclusion, this study demonstrates that the duration of key stages in the early and later life of the mother (and to a lesser extent the father) are significant predictors of a range of performance-related metabolic and growth-related traits of their offspring. The durations of these life history stages are largely determined by environmental conditions, with each life stage taking longer in a poorer quality of environment. While the mechanisms underlying these parent-offspring relationships are not yet known, it is possible that parents may optimise offspring traits to suit the quality of environment they themselves experienced as a juvenile, so that they potentially have the capacity to influence the life history trajectory of their offspring.

Chapter Four: Prior Resource Availability and Life History Origin Affect Competitive Behaviour in Territorial Disputes

*Note: A version of this chapter has been accepted as a manuscript in the journal Behavioral Ecology.

4.1 Introduction

Dominance hierarchies generally result in dominant individuals gaining preferential access to food, shelter or mates. Differences in dominance-related traits (e.g. aggression, food acquisition, competitive ability etc.) have been linked to variation in growth (Vøllestad & Quinn 2003; Adriaenssens & Johnsson 2011) and life history strategies (Biro & Stamps 2008; Chapman et al. 2011) but may be dependent upon condition. For example Van Leeuwen et al. (2011) demonstrated that dominant individuals experienced higher growth rates than subordinates when food was abundant but had lower growth rates than subordinates at low food as dominant individuals, being larger, became more constrained by the food availability in their habitat.

Maximising energy intake (food consumption) without also increasing energy expenditure (through costs of foraging, defending territories and movement) is a problem faced by many organisms. If habitats differ predictably in their productivity, this net rate of energy intake is likely to have important implications as to whether it is more profitable for an individual to remain in a locality (resident approach) or leave (migratory approach; Gross et al. 1988). There will be individual variation in the net rates of energy intake in a given habitat, and as a result the trade-off between potential net energy intake as a resident or a migrant also differs between individuals. Partial migration, in which some individuals of a population migrate and others remain sedentary is a phenomenon that occurs across a wide range of taxa (see reviews in: Chapman et al. 2011; 2012; Dodson et al. 2013). The commonest form of this intraspecific variation in movement patterns is non-breeding partial migration (sensu Chapman et al. 2011), where migrants and residents breed sympatrically but forage in different habitats. There have been many hypothesized explanations for this category of migration, including intraspecific competition for limited food resources, predation risk trade-offs and intraspecific niche diversity (see Chapman et al. 2011).

However, there is a lack of hard evidence of the factors that pre-dispose particular individuals to one or the other strategy. Those that by chance have the advantage of greater

access to resources may be less liable to migrate (Sandell & Smith 1991). Similarly, residency may be more likely in those encountering a low local density of competitors (as has been demonstrated experimentally in red-spotted newts *Notophthalmus viridescens* by Grayson & Wilbur (2009)), or those experiencing a higher food supply (as in salmonid fish: Olsson & Greenberg 2004; Olsson et al. 2006; Wysujack et al. 2009). Body size may also affect the selection pressures for/against migration, since larger individuals may generally have less to gain from migration (Dodson et al. 2013, though see Brodersen et al, 2008 for an exception) since they usually have the advantages of a higher competitive ability and/or lower risk of starvation and predation (Chapman et al. 2011).

One area which has received a considerable amount of attention in explaining patterns of partial migration, mainly in birds, has focused on the role of dominance-related traits and body size (Gauthereaux 1982; Nilsson et al. 2008). More dominant individuals tend to out-compete subordinates for limited food and breeding resources, which in turn forces subordinates to migrate in search of more profitable environments (Gauthreaux 1982). While this hypothesis has been supported in several studies (Lundberg 1985, Nilsson et al. 2008) it has been rejected by others (Rogers et al. 1989; Boyle 2008), indicating that the role of dominance status (and hence often body size) in explaining patterns of life history diversity may be context dependent. In particular the environmental conditions that are experienced at the time and whether or not an individual's migratory tendency is fixed (i.e. determined by its parents through genetic or parental effects, so that offspring of migrants are themselves migratory), as demonstrated by Berthold (1988) and Berthold & Pulido (1994) for migratory tendency and migration distance in the Blackcap *Sylvia atricapilla*, or flexible (condition-dependent; Brodersen et al. 2008).

A well-documented example of partial migration is the brown trout *Salmo trutta*, a polymorphic species that adopts a continuum of life history strategies, with the two most common being freshwater-resident and anadromous migrant (i.e. born in freshwater but growing at sea, before returning to fresh water to spawn). The two ecotypes can occur in sympatry, possibly derived from a single gene pool, with anadromous and freshwater-resident adults having the ability to interbreed and both being able to produce offspring apparently capable of adopting either life history, depending on food availability (Olsson et al. 2006; Wysujack et al. 2009; O'Neal & Stanford 2011), although the probability of adopting either life history may vary depending on parentage (as demonstrated for a closely related species the rainbow trout (*Oncorhynchus mykiss*; Nichols et al. 2008).

While it is likely that genetics interacts with growth history, current body size and physiological condition to determine whether or not the animal migrates, there may also be a role for inherent differences in dominance-related traits independent of body size. Soon after emergence from the nest, brown trout fry rapidly establish dominance hierarchies that are temporally stable (Johnsson & Forser 2002; Jonsson & Jonsson 2010) and engage in intra- and inter-specific competition for preferred feeding territories (Lahti et al. 2002; Klemetsen et al. 2003). It is generally accepted that dominance is advantageous since it gives preferential access to food (Alanära & Brännas 1996) and so facilitates increased growth (Klemetsen et al. 2003), including in the wild (Höjesjö et al. 2002). The degree to which an individual competes for a territory may also depend on its previous experience. In brown trout fry it has been shown that both previous rearing density (Sundström et al. 2003) and habitat preferences (Johnsson et al. 2000) influence territorial competitiveness, which may also be influenced by the fish's nutritional state (Johnsson et al. 1996)

Therefore given that one of the drivers for migration is the relative rates of resource acquisition in different habitats that is likely dependent on traits associated with dominance of the individual we test 1) whether size-matched juvenile offspring of freshwater resident and anadromous brown trout differ in dominance related traits (food acquisition, spatial position, colour and aggression) in dyadic contests when competing for feeding territories in a semi-natural stream channel and 2) whether differences in these dominance related traits depends on the level of food availability that individuals have experienced earlier in life. Differences in relative dominance traits of offspring from alternative life histories would provide evidence for parental effects (genetic and/or non-genetic) as a potential mechanism perpetuating the maintenance of alternative life histories in partially migrating populations, whereas an effect of prior food availability would indicate that dominance related traits could be influenced by non-genetic differences in the quality of their early environment.

4.2 Methods

4.2.1 Broodstock Collection

Twenty-four mature freshwater-resident (12 male and 12 female) and 14 anadromous (7 male and 7 female) brown trout were captured using electrofishing on 11 and 23 October 2013 from two neighbouring sub-tributaries of the River Tweed, Scotland. Freshwater-resident trout were collected from above an impassable dam on the Whiteadder River (55° 88'N, 2°57'W) while the anadromous trout were collected from the College

Burn (55° 77'N, 2°18'W). Fish were classified as freshwater-resident or anadromous based on existing knowledge of the composition of the populations present from previous scale readings, size and colouration (Eek & Bohlin 1997): freshwater-resident fish were smaller and dark brown in colour with red spots, while anadromous fish were larger and silvery-grey in colour with black spots. Both ecotypes were transported to the Belhaven Trout Company, Scotland, where they were held separately in two round 1530 L aluminum tanks supplied with 8.1 ± 0.4 °C (mean±SD) well water under ambient photoperiod and assessed every three days for ripeness.

Ripe fish were anaesthetised, blotted dry, and their eggs or sperm extruded by abdominal massage. Eggs were fertilised with sperm from a haphazardly-chosen male of the same life history origin to create 12 full sibling freshwater-resident families and 7 full sibling anadromous families. Freshwater-resident and anadromous fish were artificially spawned from 3 November - 29 November and 17 November - 4 December 2013 respectively.

4.2.2 Egg Rearing, Hatching and Fish Husbandry

Each family of eggs was housed separately in a plastic mesh egg basket, placed in one of two (1m X 3m X 0.4m) rearing troughs supplied with well water and covered with dark plastic sheeting to ensure eggs were in complete darkness. Water temperature during incubation was 8.1 ± 0.4 °C and was recorded daily along with any dead eggs which were carefully removed.

Eggs were checked daily for hatching; those from freshwater-resident and anadromous fish hatched from 19 December 2013 - 17 January 2014 and 30 December 2013 - 24 January 2014 respectively. Once eggs began to hatch, the newly emerged offspring (alevins) were separated from the remaining eggs and gently placed into a small mesh basket (one per family) located in the same two troughs as the egg baskets.

On 31 January 2014 alevins (i.e. hatched embryos still dependent on the yolk sac for nutrition) were transported to the Scottish Centre for Ecology and the Natural Environment, Scotland. Families were housed separately in 15 L (50cm X 30cm X 15cm) clear plastic aquaria on a partial recirculation system at a constant temperature of 9.2 ± 0.2 °C (mean±SD) and simulated ambient photoperiod. The aquaria each contained a single air stone and were supplied with water pumped directly from Loch Lomond, which was first

treated with an ozone generator (Sander S1000, Germany) before being discharged into a large sump. Water from the sump was pumped through an in-line 110W UV steriliser (Tropical Marine Center (TMC), Manchester, UK) before entering the aquaria. Return water was gravity fed into a large free standing filter before being discharged back into the main sump. Fish were monitored daily and any mortalities removed. On 3 March 2014, once all fish had used up their yolk sac and began feeding on exogenous food consistently, equal numbers of offspring from each family were haphazardly assigned into twelve round 121 l (r=40cm, h=24cm) tanks (keeping parental type discrete), with six tanks per parental type and 200 fish per tank. Tanks were supplied with water pumped directly from Loch Lomond and held under simulated ambient photoperiod and temperature (12.3 $\pm$ 1.7 °C (mean$\pm$SD)). Tanks were assigned to one of three food treatments (giving 2 replicate tanks per food treatment per offspring ecotype) and fed twice daily on a standard commercial salmon pellet (Biomar, Aarhus, Denmark) for the remainder of the experiment. The three food treatments were high food (approximately 4.3 % body wt. day^{-1}), mid food (approximately 2% body wt. day^{-1}) and low food (0.7% body wt. day^{-1}). High food treatments were based on feed amounts for a given size of fish and temperature recommended by Biomar (Aarhus, Denmark) for maximum growth in trout, with low food and mid food treatments selected to achieve growth rates slightly above maintenance and intermediary between maximal and minimal growth rates respectively, similar to Wysujack et al. (2009).

4.2.3 Methodology for Testing Relative Dominance

The fish were tested for dominance between 25 Sept. 2014 and 15 Nov. 2014, when they were approximately ~7 months old. All trials were conducted in an oval shaped artificial stream channel located at the Scottish Centre for Ecology and the Natural Environment, Scotland (Fig.4.1; next page).

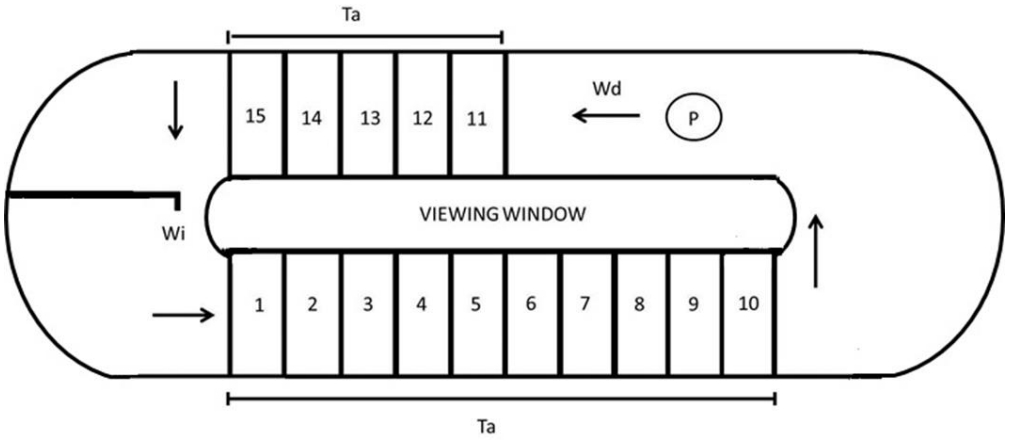

Fig.4.1. Schematic diagram of the experimental stream channel used in this experiment with the straight sections of stream channel divided into 15 equally sized test arenas (Ta); arrows indicate water direction (Wd), with the pump (P) and water inflow (Wi).

Water to the stream channel was pumped at approximately 15 l min^{-1} directly from Loch Lomond, and so was at the same temperature as in the fish's rearing tanks. Straight sections of the stream channel contained a clear glass window which ran the entire length of the channel to allow observations to be carried out. A canopy of dark sheeting between the straight sections of the stream channel allowed for observations of fish to be carried out from a concealed location and increased the contrast of light to further prevent detection of observer by fish. Both arms were fitted with plastic meshed transverse dividers mounted on wooden frames to give a total of 15 smaller compartments (60cm X 60 cm X 60 cm; Fig.4.1). The substratum was homogenously landscaped with gravel. A 10cm X 5cm rock was placed in the middle of each compartment to produce a single preferred position (so increasing the likelihood of competition between the two fish; Metcalfe et al. 2003). An electric pump ensured relatively uniform velocity (~0.15 ms^{-1}) throughout the artificial stream channel.

All trials used a single pair (one freshwater-resident and one anadromous parental type) of fish from the same food treatment in each compartment. Fish were haphazardly selected from the holding tanks, anaesthetised and sized-matched within pairs for fork length ($\pm$0.6 mm; Fig. 4.2A) and mass ($\pm$ 0.13 g; Fig. 4.2B).

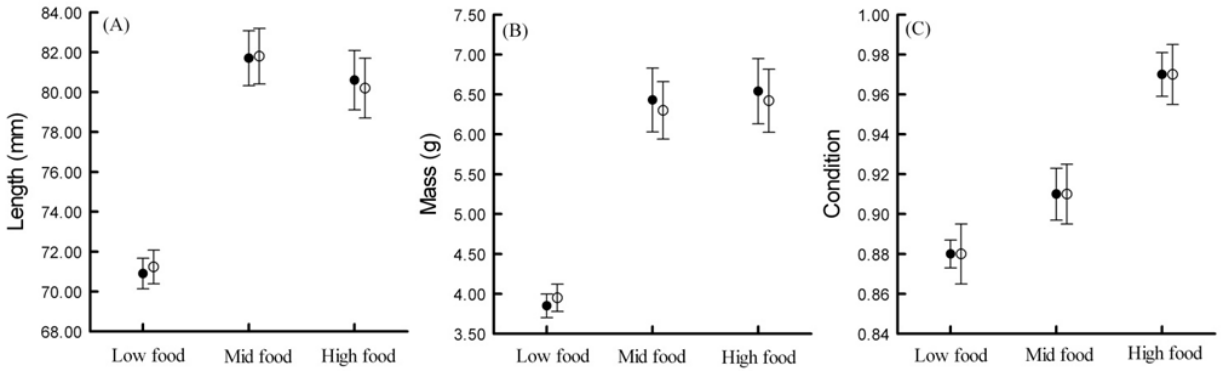

Fig.4.2. The mean (±SE) length (A), mass (B) and relative condition (C) of offspring of freshwater-resident (closed circles) and anadromous (open circles) parents used in the dominance trials. Data shown separately for fish from low, mid and high food treatments. Note that fish were selected to be size-matched within food treatments, but differences between food treatments reflect effects of treatment on growth and condition.

Once sized-matched, one of the two parental types in a pair (type alternated between compartments to prevent any bias due to tagging) was given an alcian blue dyemark on the dorsal fin prior to the experimental trial. The two fish were then released into a compartment of the stream channel and allowed to acclimate for two days prior to a two day period of behavioral observations. Because the artificial stream channel was divided into 15 compartments we were able to run 15 trials simultaneously. All 15 trials in any one run of the experiment were conducted with pairs of fish from the same food treatment. Fish were fed periodically during the two day acclimation period by flushing a single food pellet through a plastic pipe so that it dropped into the centre of the stream at the upstream end of each compartment. All procedures were carried out under the approval of the UK Home Office under project no. 60/4292.

4.2.4 Data Collection

Four observation sessions were conducted daily (09:00, 11:00, 13:00, 15:00 h) over the course of two days for each pair of fish. Each observation session consisted of first visual recordings of aggression, colour, and spatial position for each pair during a 3 min period. After the 3 min period a single food pellet was introduced (as above) into the upstream part of the compartment and a record made of the fish that obtained it. Then a second recording was made of colour and spatial position, followed by a second recording of any aggression over a 3 min period.

Aggression was scored as the total number of aggressive behavioral acts (categorized as fin nips, chases, bites, charges and dorsal displays) exhibited by each individual (Adams et al. 1995) during the 3 min observational periods.

Body colouration is a reliable indicator of social stress in juvenile salmonids, with more subordinate individuals being darker (O'Connor et al. 1999; Kaspersson et al. 2010). Colouration was assessed visually in each fish on a three point integer scale, with brighter fish receiving a score of +1 and darker ones a score of -1. Because colouration was scored twice per trial (before and after feeding) and summed across all 8 observation sessions, a fish's total score for body colouration could range from -16 to +16.

Spatial position was assessed using a protocol developed from earlier research on juvenile salmonids (Metcalfe et al. 2003; Burton et al. 2011). Spatial positions were quantified in three dimensions by marking each axis of a compartment into three equal lengths, using equally distributed marbles on the substrate and marks on the inside viewing window. These marks defined 27 (i.e. 3 x 3 x 3) equal-sized cuboid zones. Since previous research has shown that dominant salmonids occupy central-rear positions, often just above the substrate and behind an object (Metcalfe et al. 2003; Burton et al. 2011), we gave fish that occupied zones in the lower two thirds of the water column at the centre and back of the compartment a score of +1, while those in zones in the corners of the compartment were given a score of -1 and those in all other positions a score of 0 (Burton et al. 2011). Furthermore fish that were observed to be resting against the downstream meshed divider were given an additional score of -1, as were those resting on the substrate. Because spatial position was scored twice per trial (before and after feeding) and summed across all 8 observation sessions, a fish's total score for spatial position could range from -48 (if it was always resting on the gravel and against the mesh divider in a corner position) to +16 (if it was consistently in a middle position and not touching the mesh or substrate).

The ability to acquire food under competition was scored with reference to the fate of the single food pellets introduced in each observation session. Individuals that made no attempt at acquiring the pellet were given a score of 0, those that attempted but failed to acquire it were given a score of +1 and the fish that succeeded in getting the pellet was given a score of +2. Total scores for competitive ability were summed across all 8 observational periods and ranged from 0 (if an individual made no attempts at feeding) to

+16 (if an individual was successful in acquiring the food pellet during every observational period).

Once observations were complete, the fish were anaesthetised, adipose fin clipped (to ensure they weren't used again) and returned to their holding tank (to be used as part of an on-going experiment examining the potential factors driving anadromous migration in brown trout) and new fish selected; each fish was therefore only used once. A total of 30 pairs from each food treatment were tested during the course of the experiment.

4.2.5 Calculations and Data Analyses

Offspring condition was calculated to illustrate differences between food treatments. Relative condition factor, K_{rel}, was calculated according to Froese (2006) using the following equation:

$$K_{rel} = W/aL^b$$

where W is the mass of the individual offspring in grams, L is the fork length in millimetres and a and b are the exponential form of the intercept and slope derived from the regression of mass vs. length plotted on double logarithmic axes for all the offspring combined. These values in addition to the mass and length measurements were used separately as the response variable in a linear model (LM) with parental type and food treatment as explanatory variables. Furthermore condition, mass and length were analysed using paired t-tests to check that the pairs in dyadic contests did not differ systematically between parental types in condition, mass or length.

Individual scores for position, food acquisition, colour and aggression were normalised prior to analysis (by subtracting the mean score of all 180 fish from each individual's score and dividing this value by the standard deviation for all fish; Burton et al. 2011). These normalised scores were then used as the response variable in a generalized linear mixed model (GLMM) with parental type and food treatment as explanatory variables and pair ID as a random factor to control for the non-independence of measures. Associations between normalised scores of the four measures (aggression, colour, spatial location and food acquisition) were described using Pearson correlations and then summarised with a principal components analysis (PCA) summarising all four behaviours as a general index of fish dominance. Principal Component 1 (PC1) was found to be highly associated with each of these four separate measurements, with more positive scores indicating more dominant individuals. PC1 scores were therefore analysed using a GLMM

with parental type and food treatment as explanatory variables and pair ID as a random factor. All GLMM models initially included all two way interactions. Variance inflation factors (VIF's) for all explanatory variables were calculated prior to analysis; all VIF's were less than 3, indicating that collinearity among explanatory variables was unlikely to have affected our analyses (Zuur et al. 2009). All statistical models were validated by visual inspection of residual plots which did not reveal any obvious deviations from homoscedasticity or normality. Likelihood ratio tests comparing models with and without a given term were used to sequentially compare model fit; models were progressively simplified provided that any increase in the log-likelihood ratio statistic was not significant ($p > 0.05$) and checked using AIC criterion to validate the model of best fit. Initial analyses combined both parental types and all three treatments. If a significant food by parental type interaction was found, data for each food treatment was analysed separately using the above procedure and parental type as a fixed effect. Analyses were conducted using the R version 3.0.1 statistical software (R Core Team, 2013) and the lme4 function (Bates, Maechler & Bolker, 2012).

4.3 Results

4.3.1 Growth and Condition of Fish

There was a significant effect of prior rearing environment on offspring length and mass ($F_{(2,176)} = 42.60$, $p < 0.001$; $F_{(2,176)} = 38.72$, $p < 0.001$; Fig. 4. 2A and 4.2 B; pg.52), with pairs drawn from the low food treatments being shorter and lighter compared to those from high (Tukey, $p<0.001$) and mid food treatments (Tukey, $p<0.001$). There was no significant difference in length or mass between mid and high food treatments (Tukey, length: $p=0.55$; mass: $p=0.94$). A significant effect of prior rearing environment on relative condition factor ($F_{(2,176)} = 22.78$, $p < 0.001$) was primarily driven by a lower condition in fish drawn from the low food treatments and mid food treatments compared to those from high (Tukey, $p<0.001$), since there was no significant difference in condition between mid and low food treatments (Tukey, $p=0.19$). The size-matching of fish within each food treatment was successful, since there were no differences between parental types in length, mass or condition ($t_{89}=-0.09$, $p=0.93$; $t_{89}=0.83$, $p=0.41$; $t_{89}=1.33$, $p=0.19$; Fig. 4.2; pg. 52).

4.3.2 Aggression

There was no significant interaction between parental type and prior rearing environment ($\chi^2=3.67$, df= 2, $p=0.16$) on aggression score. There was however, a significant effect of prior rearing environment on aggression score ($\chi^2=17.25$, df= 2,

p<0.001; Fig. 4.3A), with pairs drawn from the low food treatments having a lower aggression score compared to those from high (Tukey, p<0.001) or mid food treatments (Tukey, p<0.01).

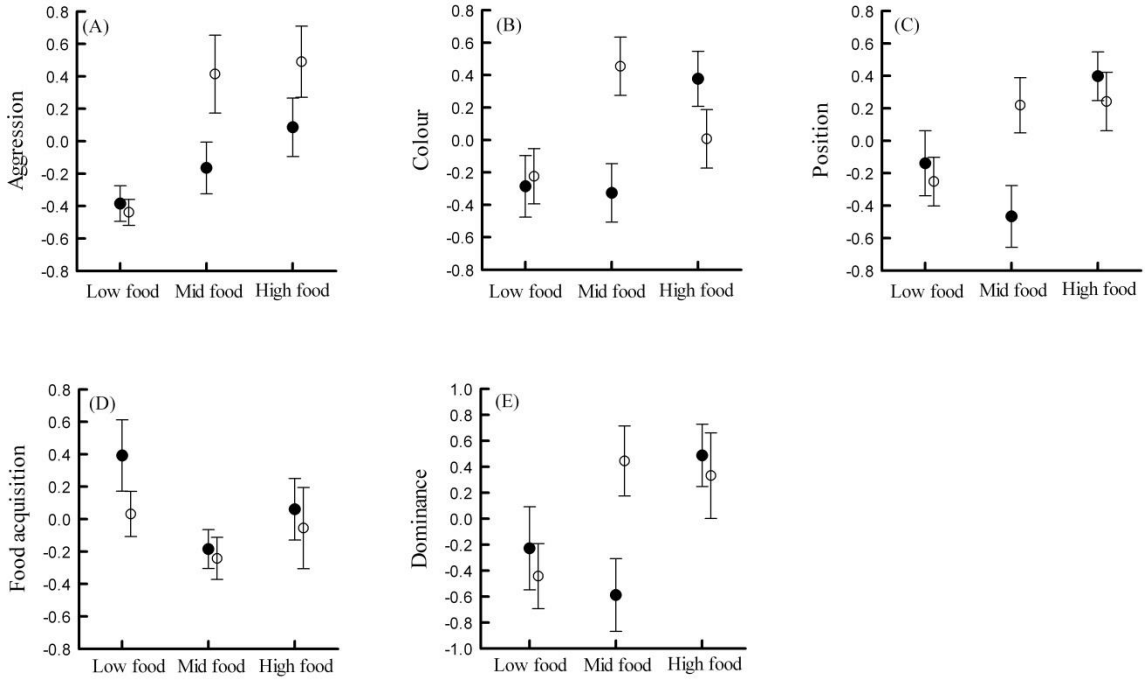

Fig.4.3. The mean (±SE) scores for aggression (A), colour (B), position (C), food acquisition (D) and dominance (E) of offspring of freshwater-resident (closed circles) and anadromous (open circles) parents, plotted separately for pairs from low, mid and high food treatments. Data are plotted as z-scores except for (E) which shows scores for the first principal component in a PCA of the other four variables (A-D). See text for statistical analysis.

There was also an effect of parental type (χ^2=12.43, df= 2, p<0.001), with anadromous offspring having a significantly higher aggression score compared to freshwater-resident offspring. Analysis of the food treatments separately revealed that this overall effect was driven primarily by the significant difference in aggression between the two parental types in mid food pairs (χ^2=4.16, df= 1, p=0.041), as there was no difference in aggression between parental types from the low (χ^2=0.16, df= 1, p=0.069) or high food treatments (χ^2=2.37, df= 1, p=0.12).

4.3.3 Colour

There was a significant interaction between parental type and prior rearing environment (χ^2=10.96, df= 2, p=0.004), indicating that the colour of the freshwater-

resident and anadromous offspring differed depending on their prior rearing environment. Analysis of the food treatments separately revealed a significant difference in colouration between the two parental types in pairs of mid food (χ^2=9.08, df= 1, p=0.003; Fig. 4.3B; see previous page) and high food (χ^2=2.38, df= 1, p=0.012) treatment fish , with freshwater-resident offspring having a more subordinate coloration, whereas there were no differences in colour between parental types in pairs from the low food treatments (χ^2=0.063, df= 1, p=0.080).

4.3.4 Position

There was a significant interaction between parental type and prior rearing environment (χ^2=7.32, df= 2, p=0.026) indicating that the position occupied by freshwater-resident and anadromous offspring differed depending on their prior rearing environment. Analysis of the food treatments separately again revealed a significant difference between the two parental types in the mid food treatment (χ^2=6.88, df= 1, p=0.009; Fig. 4.3C; see previous page), with anadromous offspring in that treatment occupying higher scoring positions compared to freshwater-resident offspring; there were no differences in spatial position between parental types in either the high (χ^2=0.46, df= 1, p=0.50) or low food pairs (χ^2=0.20, df= 1, p=0.65).

4.3.5 Food Acquisition

There was no significant interaction between parental type and prior rearing environment (χ^2=0.812, df= 1, p=0.67) or an effect of parental type or prior rearing environment on food acquisition score (χ^2=5.58, df= 1, p=0.061; χ^2=1.48, df= 1, p=0.22; Fig. 4.3D; see previous page).

4.3.6 Dominance

Pearson correlations indicated that the four traits recorded in the behavioural observations were significantly correlated (Table 4.1; next page).

Behavioural traits				
	Aggression	Colour	Position	PC1
Aggression	-	-	-	0.45
Colour	0.50	-	-	0.55
Position	0.49	0.73	-	0.54
Food acquisition	0.37	0.50	0.49	0.45

Table 4.1. Pairwise Pearson's correlation coefficients and PC1 coefficients from PCA analysis, for all four behavioural traits observed. All four traits were significantly correlated with one another (df=178, $p<0.001$), with the first Principal Component summarising 64% of the variation.

Principal component analysis indicated that they could successfully be combined into a single PC that summarized 64% of the variation (Table 1), with high PC1 scores indicating individuals with high aggression, pale colouration, favourable spatial positions and high scores for food acquisition; PC1 was therefore defined as a composite measure of dominance. There was a significant interaction between parental type and prior rearing environment (χ^2=6.23, df= 2, p=0.04), indicating that the relative dominance score of freshwater-resident and anadromous offspring depended on their prior rearing environment. Analysis of the food treatments separately revealed a significant difference in dominance between parental types in pairs of mid food treatment fish (χ^2=6.91, df= 1, p=0.009; Fig. 4.3E; see page 56), with offspring of anadromous fish having a higher dominance score than those of freshwater-residents, but there were no differences in dominance score between parental types in pairs from either the high (χ^2=0.147, df= 1, p=0.70) or low food treatments (χ^2=0.29, df= 1, p=0.59).

4.4 Discussion

By rearing offspring from parents with contrasting life histories under different food regimes we have demonstrated that both the effect of migration history of the parents and offspring rearing environment influenced dominance status in competition for feeding territories. Interestingly, we found that anadromous offspring scored consistently higher in position, aggression, colour and overall dominance than offspring of freshwater residents when fish had previously experienced an intermediate level of food availability, but the

two offspring types behaved similarly when reared on low or high food rations. When reared on low food rations both parental types occupied relatively poorer spatial positions and showed very low (= more subordinate) scores for colour, aggression and overall dominance. In contrast, if they had previously experienced high food availability they were observed to occupy more favourable spatial positions, were more aggressive and had more dominant colouration.

The positive relationship observed between correlates of dominance and rearing environment suggests that the foraging and territorial strategy adopted is dependent on the individual's previous experience of the profitability of the environment. It has previously been argued that the strength of territorial defence should depend on resource availability, with greatest resource defence at intermediate levels (Myers et al. 1979; Toobaie & Grant 2013). Individuals from the low food treatment, which were in poorer average body condition, may have been less able to engage in costly aggressive interactions (Johnsson et al. 1996). As a result they were likely adopting an "energy minimising" or "sit and wait" strategy, whereby individuals sacrificed the option of obtaining a territory in a good feeding position to avoid the energetic costs of both swimming against the water flow and potential battles over territories (as seen by the low aggression scores in this treatment group). Since investment in territorial aggression can be costly for growth (Vøllestad & Quinn 2003), minimisation of costs may have been the best strategy for these fish (Metcalfe 1986). While these fish showed less inclination to establish a feeding territory, they nonetheless tended to acquire more food than pairs from the higher food treatments, suggesting that short-term gain was more important than the long-term benefits of a territory. In contrast, individuals from the high food treatment, which were in better body condition, tended to adopt a more active and aggressive strategy (i.e. competing for the best foraging position in the centre of the water column) that may be geared toward longer-term access to food.

The differences in foraging and competitive strategies based on an individual's prior experience of access to food that were observed in our study have parallels in the study by Sundström et al. (2003), which showed that brown trout reared in a hatchery were more aggressive in territorial defence than wild-reared conspecifics. These findings may help to explain inconsistences in the relationship between dominance status and growth found in other experimental studies. For example, juvenile Atlantic salmon with higher social status have been shown to have higher grow rates than subordinates when fed ad

libitum from a point source in the laboratory (Metcalfe et al. 1989) but have similar or lower growth rates relative to subordinates when food predictability decreases (Huntingford & Leaniz 1997; Höjesjö et al. 2002; Reid et al. 2012).

Although traits related to dominance did not differ between offspring from freshwater-resident and anadromous parents if they had been reared on high or low food rations, there was a significant difference in dominance if they had been reared at an intermediate food level (with offspring from anadromous parents behaving in a more dominant and competitive manner). This asymmetry suggests that the two parental types differ in the environmental threshold at which they adopt a more competitive and aggressive foraging strategy, with offspring from anadromous parents switching to territorial behaviour at a lower food level. One possibility for the observed difference between parental types is inherent differences in foraging motivation driven by asymmetries in physiology or projected life history. Differences in motivational state linked to hunger have been shown to increase aggression and strengthen social interactions in birds (Andersson & Ahlund 1991) and fishes (Dill et al. 1981; Johnsson et al. 1996), and it may be that the offspring of anadromous parents have either a different food requirement or threshold for territoriality, leading to the observed differences in aggressiveness between parental types on mid food rations.

Another possibility for the parental type by food level interactions could be the timing of the experiment with respect to whether or not individuals were destined to migrate. Given their size at the time of the experiment, the earliest that any of the experimental fish would migrate to sea would be in the following spring. Morinville & Rasmussen (2003) demonstrated that individual migrant brook trout (*Salvelinus fontinalis*) had higher food consumption rates and lower growth efficiencies in the year leading up to migration compared to sympatric resident brook trout, suggesting that the migrant brook trout had higher metabolic costs. Furthermore, Thorpe et al. (1998) proposed that the decision to migrate in the closely-related Atlantic salmon occurs soon after mid-summer (around the time of this study): fish that fall below their individual threshold for growth rate at this time subsequently decline in appetite, cease growth and defer migration, while those individuals who are above the growth threshold maintain their appetite throughout late summer and autumn and undergo migration the following spring. If this pattern of migration holds for brown trout, then all fish in the low food treatment may have already failed to reach the threshold triggering migration. However, if the tendency to migrate is

partially genetically determined (Berthold 1988; Berthold & Pulido 1994), then under the mid food treatment offspring of migrants might have had a lower growth threshold triggering migration, so would have a greater motivation to acquire feeding territories so as to be able to maintain their growth rate. Meanwhile all fish reared on the high food treatment may have passed their respective thresholds, leading to aggressive and dominant behavior being expressed by both parental types in preparation for migration the following spring.

One potential caveat to our study is that we were unable to determine whether the differences between offspring behaviour were primarily due to genetic or maternal effects, but this would be difficult to establish given that the resident-anadromous dichotomy by its very nature prevents the use of the standard approach of rearing the parents in a common garden to rule out maternal effects.

In conclusion, the results of this study demonstrate that both the life history strategy of the parents and the rearing environment of the offspring may have a significant effect on a range of dominance related traits. Therefore we suggest that inherent differences in dominance-related traits, when coupled with spatial variation in environmental productivity, may play a significant role in the perpetuation of non-breeding partial migration within populations.

Chapter Five: Offspring of Migratory Trout Show Less Diet-induced Flexibility in Morphology and Growth than Offspring of Residents

*Note: A version of this chapter has been accepted as a manuscript in the Canadian Journal of Fisheries and Aquatic Sciences.

5.1Introduction

Phenotypic flexibility, an organism's ability to match its morphology and physiology to current environmental conditions, is fundamental to adaptability and occurs when complimentary combinations of traits change in response to environmental conditions to maximise the efficiency of resource exploitation. For example, within fish there may be changes within the lifetime of the individual animal in gill raker spacing and mouth shape to suit shifts in prey type (Schluter 1993), changes in body shape that are related to parallel changes in the velocity of water in which the fish is living (Peres-Neto & Magnan 2004) and more recently differences in SMR (the minimal maintenance metabolic rate of an ectotherm in a post-absorptive and inactive state) in response to local food availability (Van Leeuwen et al. 2011; Auer et al. 2015). While much of the research surrounding phenotypic flexibility has been focussed on explaining patterns of resource polymorphisms within species in the context of adaptive radiation and speciation, it may also help explain other ecological patterns of intraspecific variation, one of which is the phenomenon of partial migration.

Partial migration, in which members of a population differ in whether or not they undertake migrations, occurs across a wide range of taxa including invertebrates (Hansson & Hylander 2009), fish (Dodson et al. 2013), birds (Newton 2008) and mammals (Ball et al. 2001). The commonest form is non-breeding partial migration (*sensu* Chapman et al. 2011), where migrants and residents breed sympatrically but overwinter apart. There have been many hypothesised explanations for this variation in migratory pattern, including competition for resources, differences in thermal tolerances and differences in arrival times/prior residence (see Chapman et al. 2011). In all cases however, the migration can be viewed as a response to adversity (Taylor & Taylor 1977), but the degree of adversity will depend on the particular environmental conditions that are experienced at the time. For example, individuals that are of a larger body size or experiencing a higher food supply may generally have less to gain from migration (Chapman et al. 2011).

It is likely that both abiotic and biotic factors influence the decision to migrate or not, since it is potentially influenced by both genetic causes (i.e. determined by the parents through genetic or parental effects, so that offspring of migrants have a higher probability to migrate) and environmental factors (e.g. through condition-dependent migration; Brodersen et al. 2008). Berthold (1988) and Berthold & Pulido (1994) provide support for a genetic pre-disposition for migratory tendency and migration distance in the Blackcap. However, it has also been suggested that partial migration is driven by a complex interaction between the environment and genetics. In the "threshold model" the triggering of migration depends on whether or not a continuous character ("liability trait") exceeds a genetically predetermined threshold value (Chapman et al. 2011; Dodson et al. 2013). In this scenario, individuals physiologically self-evaluate their performance against this threshold (e.g. of growth rate, body size or physiological condition), with migration being dependent on whether or not the threshold is exceeded (Fleming 1996; Pulido 2011; Dodson et al. 2013).

A well-documented example of a species exhibiting partial migration is the Brown trout, a polymorphic species that adopts a continuum of life history strategies, with the two most common being freshwater-resident and anadromous migrant (which migrates to sea as a juvenile and returns to fresh water to spawn). The two ecotypes can occur in sympatry, possibly derived from a single gene pool, with both anadromous and freshwater-resident adults having the ability to interbreed and produce offspring capable of adopting either life history (Wysujack et al. 2009; O'Neal & Stanford 2011). Freshwater-resident and anadromous trout appear indistinguishable during early life, and it is presumed that they only become separable when after one or more years the migrants turn silver in colour in preparation for entry to sea water ('smolting'; Jonsson 1985). Jonsson (1985) proposed that migrant brown trout are made up of the slower growing individuals in a population, which migrate in search of more productive habitats. It has also been suggested that metabolic constraints play an important role in determining physiological state and thus migration probability. The fish are often found in oligotrophic habitats in fresh water (e.g. upland temperate lakes and streams), and in this low food environment individuals with a lower growth efficiency, higher food requirement and/or higher metabolic rate (i.e. energy maximisers) will become energetically constrained earlier in life compared to those with higher growth efficiency, lower food requirement and/or lower metabolic rates (efficiency maximisers; Metcalfe et al.1995; Forseth et al. 1999; Morinville & Rasmussen 2003; Rosenfeld et al. 2013). The individuals with the lower growth efficiencies and/or higher

metabolic rates may therefore migrate in search of more productive habitats (lakes, oceans) to meet their outstanding metabolic needs.

It is likely that genetics interacts with growth history, current body size and physiological condition to determine whether or not the animal reaches the threshold that triggers migration. However, there may also be a role for the morphological and physiological flexibility of the organism (i.e. its phenotypic flexibility). For example many species of fish adjust their body shape in response to diet type and water velocity to increase their efficiency of prey detection, capture and handling of prey items (Skulason & Smith 1995; Adams & Huntingford 2002) and to reduce swimming costs (Peres-Neto & Magnan 2004). Furthermore, flexibility in physiology may be equally important since individual brown trout that showed the biggest change in SMR when food availability was altered (either upwards or downwards) were recently found to have the fastest growth under the new food regime (Auer et al. 2015). Given that freshwater fluvial ecosystems are often regarded as being stochastic and that the decision to migrate is likely based on a cumulative assessment of performance over a range of environmental conditions experienced to date (i.e. a timespan of several years), it is possible that differences in the phenotypic flexibility of the individual may be more important in determining growth performance, and thus explaining patterns of partial migration, than whether it has a consistently "high" or "low" value for traits or conditions of interest. Therefore individuals who are more able to match morphology and physiology to current environmental conditions, and therefore to maximise growth (or minimise their energetic costs), may be more suited to freshwater fluvial habitats compared to less phenotypically flexible individuals who may be more suited to more homogenous habitats such as large lakes and oceans. If true, then offspring of freshwater-resident parents might be more likely to exhibit plasticity in early life than those of anadromous brown trout.

To explore these issues, we reared brown trout offspring from eggs of known parentage (i.e. freshwater-resident or anadromous) under two diets of equal energy content but potentially differing ease of digestion (*Daphnia* and Chironomid larvae), which were then switched to test for phenotypic flexibility in both morphology and physiology, and the consequences for growth rate.

5.2 Methods

5.2.1 Broodstock Collection

Twenty-four mature freshwater-resident (12 male and 12 female) and 14 anadromous (7 male and 7 female) brown trout were captured during the breeding season using electrofishing on 11 and 23 October 2013 from two neighbouring sub-tributaries of the River Tweed, Scotland. Freshwater-resident trout were collected from above an impassable dam on the Whiteadder River (55° 88'N, 2°57'W) while the anadromous trout were collected from the College Burn (55° 77'N, 2°18'W). Adult fish were classified as freshwater-resident or anadromous based on size and colouration (Eek & Bohlin 1997): freshwater-resident fish were smaller and dark brown in colour with red spots, while anadromous fish were larger and silvery-grey in colour with black spots. Both ecotypes were transported to the Belhaven Trout Company, Scotland, where they were held separately (keeping parental ecotypes discrete) in two round 1530 L aluminum tanks supplied with 8.1 $\pm$ 0.4 °C (mean$\pm$SD) well water under ambient photoperiod and assessed every three days for ripeness.

Ripe fish were anaesthetised, blotted dry, and their eggs or sperm extruded by abdominal massage. Eggs were fertilised with sperm from a haphazardly-chosen male of the same life history origin to create 12 full sibling families from freshwater-resident parents and 7 full sibling families from anadromous parents. Freshwater-resident and anadromous fish were spawned from 3 November - 29 November and 17 November - 4 December 2013 respectively.

5.2.2 Egg Rearing, Hatching and Experimental Procedures

Each family of eggs was housed separately in a plastic mesh egg basket, placed in one of two (1m X 3m X 0.4m) rearing troughs supplied with well water and covered with dark plastic sheeting to ensure eggs were in complete darkness. Water temperature during incubation was 8.1 $\pm$ 0.4 °C and was recorded daily along with any dead eggs which were carefully removed. Eggs were checked daily for hatching; those from freshwater-resident and anadromous parents hatched from 19 December 2013 - 17 January 2014 and 30 December 2013 - 24 January 2014 respectively. Once eggs began to hatch, the newly emerged offspring (alevins) were separated from the remaining eggs and gently placed into a small mesh basket (one per family) located in the same two troughs as the egg baskets.

On 31 January 2014 alevins were transported to the Scottish Centre for Ecology and the Natural Environment, Scotland and housed in 15 L (50cm X 30cm X 15cm) clear plastic aquaria on a partial recirculation system at a constant temperature of 9.2 ± 0.2 °C (mean±SD) and simulated ambient photoperiod. The aquaria each contained a single air stone and were supplied with water pumped directly from Loch Lomond, which was first treated with an ozone generator (Sander S1000, Germany) before being discharged into a large sump. Water from the sump was pumped through an in-line 110W UV steriliser (Tropical Marine Center (TMC), Manchester, UK) before entering the aquaria. Return water was gravity fed into a large free standing filter before being discharged back into the main sump. Fish were monitored daily and any dead fish removed.

On 3 March 2014, at the time of first feeding, random selections of offspring from across families were haphazardly assigned into eight 15 L (50cm X 30cm X 15cm) clear plastic aquaria (keeping parental ecotypes discrete), with four aquaria per parental type and 10 fish per aquaria. The aquaria were placed inside a constant temperature room on a partial recirculation system at a temperature of 13.6 ± 1 °C (mean±SD), with a simulated ambient photoperiod. Fish were fed *ad libitum* several times daily by pipetting food onto the surface of each aquaria, with excess food (which was clearly visible on the bottom of the aquaria after every feed) being removed by vacuum siphon at the end of each day. Diet treatments consisted of *Daphnia* (BCUK Aquatics, Lincolnshire, England; composition: protein 5%, fat 0.7%, fibre 1%, moisture 90%) or Chironomid larvae (BCUK Aquatics, Lincolnshire, England; composition: protein 5%, fat 0.5%, fibre 0.9%, moisture 89%); diet types were supplied frozen from the manufacturer and thawed daily before feeding. It was presumed that, although the two diets had an almost identical nutritional and water content, fish would grow more slowly on the *Daphnia* treatment due to the extra costs associated with digesting and processing the hard exoskeleton of the *Daphnia* (Swaffar & O'Brien 1996) in comparison with the soft body of the Chironomid larvae. Two replicate aquaria (i.e. 20 fish) per parental type were randomly allocated to each of the two diet treatments.

On 11 June 2014 fish were anaesthetised, measured (fork length ±0.1mm; body mass ±0.0001 g), and tagged with a visible implant elastomer (Northwest Marine Technology, Inc.). They were then anaesthetised, re-measured and photographed on 2 July 2014, so that their growth rate (from 11 June to 2 July 2014) and morphological shape on their initial diets (3 March 2014 to 2 July 2014) could be measured (interval one). The SMR of all fish was then measured (see below) once over the period from 2 July 2014 to

12 July 2014. Once all fish had been subjected to metabolic measurements the two diet types were switched (12 July 2014) , so that all individuals previously fed *Daphnia* were switched to a diet of Chironomid larvae and *vice versa*. On 28 August 2014 all fish were again anaesthetised, re-measured and photographed, then their SMR recorded (measurements over the next 10 days), for assessment of growth rate, morphological shape change and metabolic rate following the diet switch (12 July to 28 August 2014; interval two). Fish were maintained on their switched diets to further evaluate the degree of shape change through later ontogeny (through to 30 September 2014, when they were again anaesthetised and photographed; interval three).

5.2.3 Measuring Standard Metabolic Rate

Aquaria were vacuum siphoned to remove food and debris the day before fish were placed in respirometry chambers. This ensured that fish were unfed for at least 28 h prior to oxygen uptake measurements, and had sufficient time to evacuate their guts; 28 h post-feeding has been shown to be adequate for the specific dynamic action (SDA) response to subside in salmonids (Cutts et al. 2002). SDA is an elevation in metabolic rate due to the increased energy demands associated with digestion, immediately following a meal (Rosenfeld et al. 2015), and is generally not considered part of SMR.

Oxygen uptake was measured over a 24 h period, from approximately 10.00 AM onwards, using intermittent flow respirometry. Individual fish were placed into 1 of 8 separate (8.0cm length, 3.4cm diameter) glass respirometry chambers. Chambers were submersed in a water bath housed inside a second constant temperature room kept at the same temperature (13.6 $\pm$ 0.5 °C across all measurements) as the tanks in which growth was measured. An air-stone in the water bath of the respirometer apparatus kept the water fully saturated with oxygen. Chambers were wrapped in dark plastic to prevent visual contact between individual fish during measurements, and all measurements were conducted in the dark to further minimise fish activity (Cutts et al. 2002). Glass respirometers and tygon tubing were used to minimise potential issues with use of plastics and oxygen permeable materials (Stevens 1992). Oxygen uptake was measured for 20 min every 45 min on a continuous 25 min "on" and 20 min "off" cycle. During the "on" cycle oxygenated water from the water bath was driven by a water pump (Eheim 300 universal, Deizisau, Germany) through each respirometer. Flow rate was regulated by adjusting the tension of a hose clamp on the outflow side of the pump tubing to prevent swimming and spontaneous behaviour during this period of flushing. After 25 minutes the pump creating

the water turnover was automatically switched off (Superpro MFRT-1 timer, Somerset, England) allowing for a decrease in oxygen concentration to be measured during the 20 min "off" period, during which a peristaltic pump (Masterflex L/S, London, England) was used to ensure adequate mixing within each respirometer. Water oxygen concentration was measured every 1s for 20 min during this time period. Oxygen concentration within the respirometer was measured using one of two oxygen meters (FireStingO_2 oxygen meter; PyroScience) each fitted with 4 oxygen probes which were placed in individual measurement chambers (Loligo systems, Tjele, Denmark) connected inline between the outlet side of each respirometer and the peristaltic pump; concentrations never dropped below 80% oxygen saturation in this experiment. Probes were calibrated daily, and rates of background oxygen consumption were subtracted from the observed values by measuring the oxygen concentration of water inside each of the respirometers in the absence of fish at the beginning and end of each measurement trial and assuming a linear decrease in oxygen concentration over the measurement period.

The rate of oxygen consumption was determined using the following equation (Ege & Krogh 1914):

$$MO_2=V_w(\Delta C_w0_2)/\Delta t$$

where V_w is the volume of water in the respirometer and associated tubing minus the volume of the fish and ΔC_w0_2 is the change in oxygen concentration of the water over time period Δt (Steffensen 1989). Oxygen concentration was calculated by correcting PO2 (partial pressure oxygen) for barometric pressure and multiplying by αO2 (μmol L^{-1} $torr^{-1}$), the solubility coefficient at the observed temperature. Standard metabolic rate was estimated by using the average of the lowest 10% of values observed during the respirometry trial (Norin 2014). Following respirometry measures all fish were anaesthetized, blotted dry and weighed to the nearest 0.0001g.

5.2.4 Morphological Measures

Lateral view photographs of all fish were taken using a Canon EOS 350D digital camera fixed to a camera stand and illuminated with two blue lights mounted on either side of the camera stand to ensure quality images for geometric morphometric analysis. For each photograph a scale reference was added to allow for the correction of shape change associated with changes in body size. Twenty consistently identifiable landmarks were digitised on each image (Figure 5.1, next page) using tpsDig and tpsUtil software (Rohlf 2006 a,b).

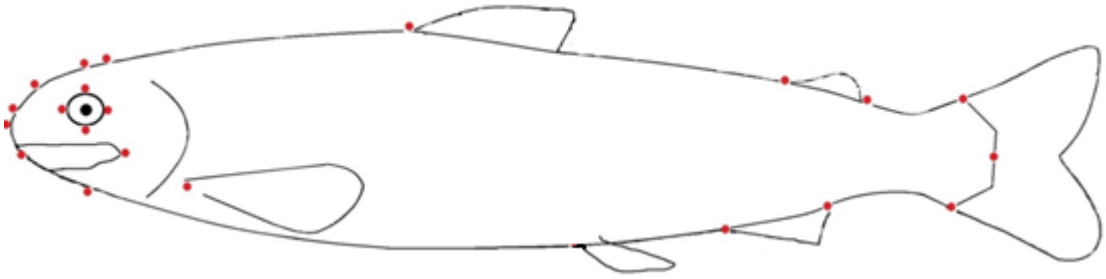

Fig.5.1. Schematic diagram of the morphological landmarks used for analysis.

Landmark configurations for each specimen were aligned, translated, rotated and scaled to a unit of centroid size by using a Procrustes superimposition using the mean shape of all the images as the starting form (Rohlf & Slice 1990). Shape change due to differences in allometry and not a response to diet type were removed (size corrected) using a multivariate, pooled, within-group regression of the Procrustes coordinates on the log centroid size of the individual (Klingenberg & McIntyre 1998). The residuals of this regression were then used for all further analysis. Canonical variate analysis was undertaken in MorphoJ to assess the effect of diet on body shape, using the average Mahalanobis distance (D) between the two diet groups from a single parent type (freshwater-resident or anadromous) for each time interval. Comparison of the changes over time in the size of D for the offspring of freshwater-resident and anadromous fish indicates the relative degree of morphological flexibility of the two offspring types.

5.2.5 Calculations and Statistical Analyses

Specific growth rates of fish (percent per day) were calculated as $100[\log_e(\text{final mass}) - \log_e(\text{initial mass})]/\text{duration}$, where duration refers to the interval between measurements (Ricker 1975). Given the large variation in fish mass and the confounding effect of mass on metabolism and growth, we used residual SMR and residual growth in subsequent analysis. These residual values were calculated as residuals from the regression of absolute oxygen consumption or growth rate (SMR or Growth) on body mass (g); in order to standardise the results I used a reference of the combined data for offspring from freshwater-resident and anadromous fish habituated to the Chironomid larvae diet (i.e. during interval one; $\log10(\text{SMR})=(1.02*\log_{10}\text{mass})+0.7576$; n=38); $\log10(\text{Growth})=(0.0116*\log_{10}\text{mass})+0.619$; n=38), plotted on double logarithmic axes. Prior to being log transformed a constant of one was added to the growth data to allow

transformation of negative growth values (since some fish on the *Daphnia* food treatments lost mass).

We used linear mixed effects models (LME) to test for the effects of diet and parental life history on growth and SMR. All LME models initially included all possible two way interactions, with aquarium tank and individual included as random factors to control for potential tank effects and non-independence of measures for individuals. Variance inflation factors (VIF's) for all explanatory variables were calculated prior to analysis; all VIF's were less than 3, indicating that collinearity among explanatory variables was unlikely to have affected the analyses (Zuur et al. 2009). Furthermore, visual inspection of residual plots did not reveal any obvious deviations from homoscedasticity or normality. Likelihood ratio tests comparing models with and without a given term were used to sequentially compare model fit; models were progressively simplified provided that any increase in the log-likelihood ratio statistic was non-significant ($p > 0.05$). Tukey and LS means tests were used to compare treatment groups. All analyses were conducted using R version 3.0.1 statistical software (R Core Team, 2013) and the lme4 function (Bates et al., 2011).

5.3 Results

5.3.1 Standard Metabolic Rate

There was no significant effect of parental type on SMR, nor of initial diet (Fig. 5.2A, next page) However overall there was a significant decrease in SMR when offspring were switched from their initial diets to their alternate diets (Fig.5.2B, next page; Tukey, all less than $p<0.001$), with fish switching from Chironomid larvae to *Daphnia* showing a greater decrease in SMR compared to those individuals that switched from *Daphnia* to Chironomid larvae (Fig. 5.2C., next page; $\chi^2=5.51$, df=1, $p<0.02$).

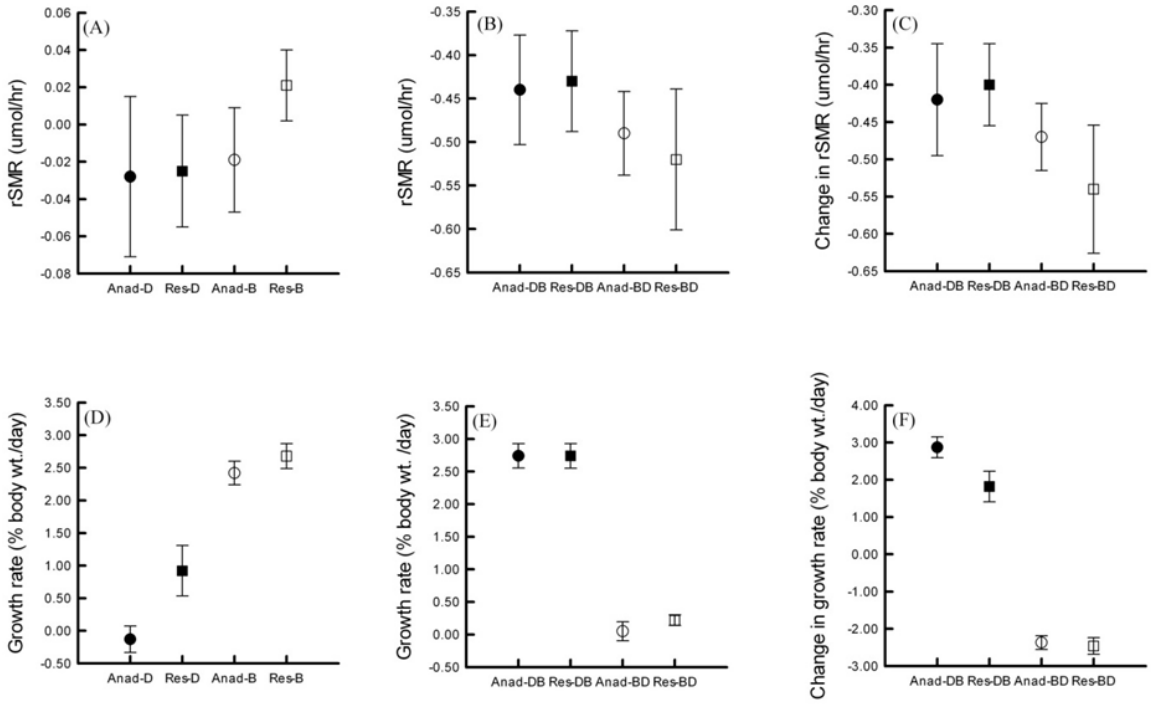

Fig.5.2. The effect of diet and parental type (squares = offspring of freshwater-resident parents (Res); circles = offspring of anadromous parents (Anad)) on standard metabolic rate (SMR) and growth rate. (a) SMR at the end of interval one of fish that had been fed since first feeding on *Daphnia* (D) and Chironomid larvae (B); (b) SMR at the end of interval two of fish that had been switched at the end of interval one from a diet of *Daphnia* to Chironomid larvae (DB; closed) or from Chironomid larvae to *Daphnia* (BD; open); note change in scale of ordinate compared to previous graph; (c) Change in SMR after the change in diet (negative values indicating a lower SMR after the switch). (d-f) Corresponding data for growth rates over (d) interval one and (e) interval two, and (f) change in growth rate after the change in diet (negative values indicating a slower growth rate after the switch). SMR and growth rates are expressed as residuals to correct for body mass. Error bars represent 95% confidence intervals. See text for statistical analysis.

5.3.2 Growth Rate

The effect of diet on growth rate depended on the parental type (Fig.5.2D, see above; χ^2=28.08, df=3, p<0.001), with offspring of freshwater-resident parents having a higher growth rate than those of anadromous parents, but only if on a diet of *Daphnia* during the first time interval (LSMEANS, p=0.04). There was no significant effect of parental type on growth when fry were feeding on Chironomid larvae (Tukey, p=0.120), or on *Daphnia* having previously been fed Chironomid larvae (Tukey, p=0.598). However fish grew faster on Chironomid larvae than on *Daphnia* (χ^2=293.1, df=3,p<0.001), and the

switch from *Daphnia* to Chironomid larvae produced a bigger increase in the growth of offspring from anadromous parents than those from freshwater-resident parents (Fig.5.2E, previous page; χ^2=6.51, df=1,p=0.01).

5.3.3 Morphometrics

There was a similar significant morphological response to diet in offspring of the two parental types (Fig. 5.3; Fig.5.4, next page), with fish initially fed on Chironomid larvae developing a rounder body and head compared to those fed on *Daphnia*, which had a more slender body and head (Fig. 5.5, next page).

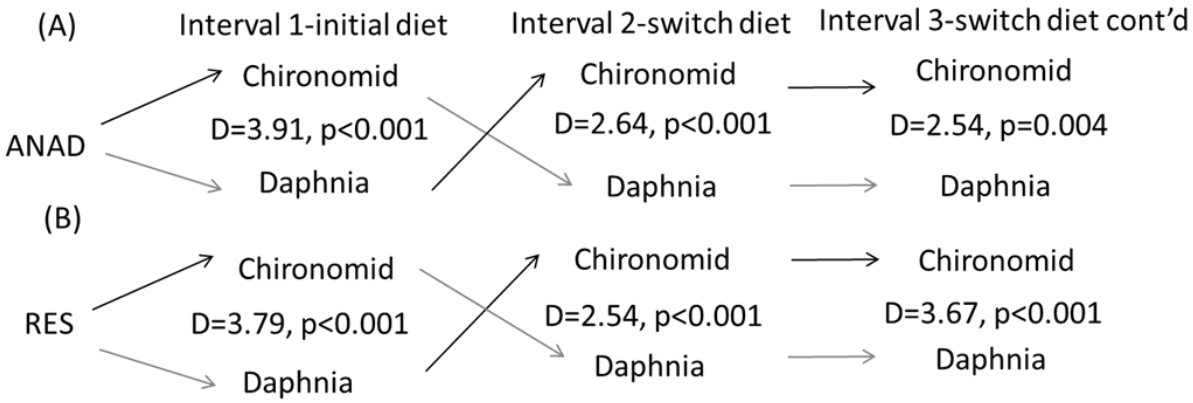

Fig. 5.3. Schematic diagram of the morphological shape response for offspring from anadromous (Anad)) and freshwater-resident (Res) parents during time periods when fish were reared on *Daphnia* (grey arrows) and Chironomid larvae (black arrows). Diets were switched at the start of interval two. Note the equivalent morphological responses to diet of the two offspring types during intervals one and two (i.e. a similar degree of initial morphological divergence between fry on the *Daphnia* and on the Chironomid larvae diets, and a similar effect of a diet switch, as measured by Mahalanobis (D) distance). However, by interval three the offspring of freshwater-resident parents showed a greater dietary-induced morphological divergence (D = 3.67) compared to those of anadromous parents (D = 2.54).

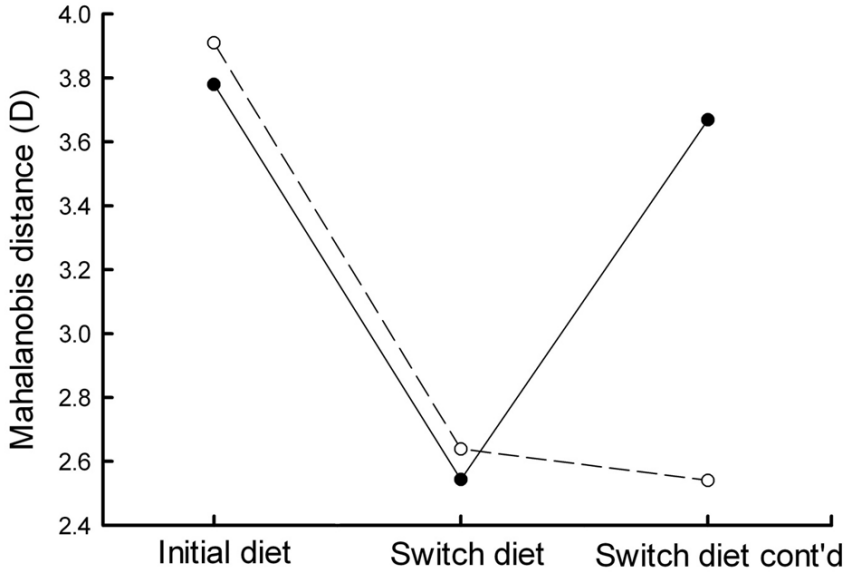

Fig.5.4. The morphological difference in shape response for offspring from anadromous (open circles; dashed line) and freshwater-resident (closed circles; solid line) parents during time periods when fish were reared on *Daphnia* and Chironomid larvae. The Mahalanobis (D) distance quantifies the difference in body shape between fish of the same parentage that were reared on the two diets. See text for further description of the analyses.

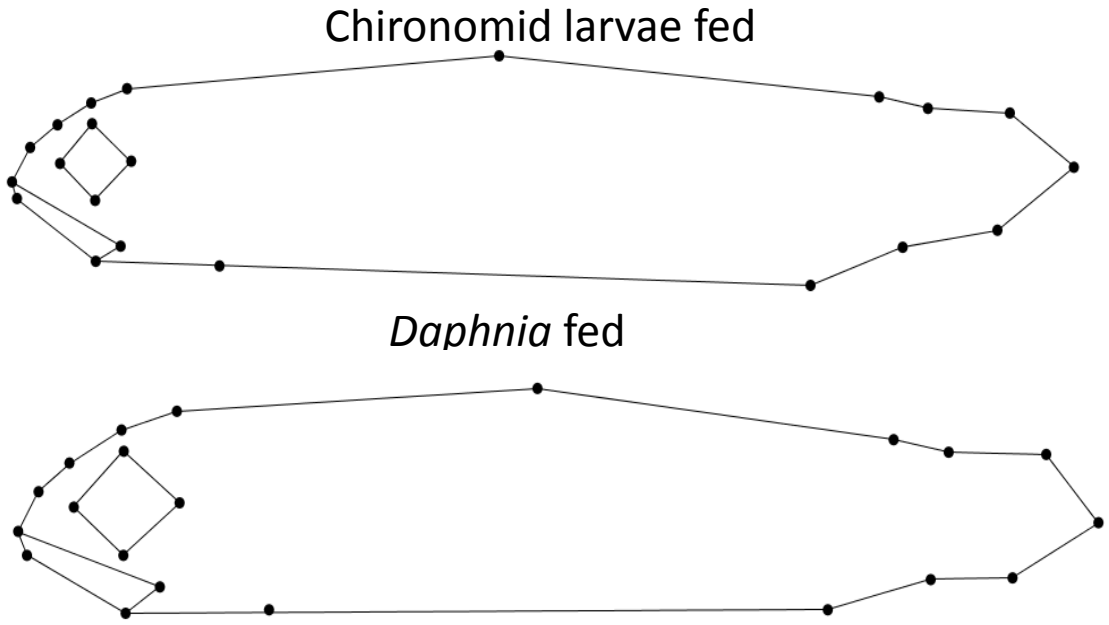

Fig.5.5. Wire frame diagrams of the morphological shape change associated with canonical variance one (CV1) from the canonical variance analysis (CVA). CV1 split groups based on diet and accounted for 36.2% of the overall variation. Note the more rounded head and body shape in the fish fed Chironomid larvae compared to those fed *Daphnia*. Analysis is based on fish morphometrics averaged across all three intervals.

When the diet was switched, the fish responded by developing the appropriate morphology (i.e. those previously fed on *Daphnia* developed a rounder body and head when switched to Chironomid larvae, and *vice versa*). The extent of the diet-induced difference in morphology was similar for offspring of the two parental types during both interval one (anadromous: Mahalanobis distance = 3.91, $p<0.0001$, freshwater-resident: Mahalanobis distance = 3.79, $p<0.0001$) and interval two (anadromous: Mahalanobis distance = 2.64, $p<0.0001$, freshwater-resident: Mahalanobis distance = 2.54, $p<0.0007$; Fig 5.3, pg. 69; Fig. 5.4, previous page). However, during interval three the offspring of freshwater-resident parents diverged more in morphology in response to diet than did those of anadromous parents (anadromous: Mahalanobis distance = 2.54, $p<0.004$, freshwater-resident: Mahalanobis distance = 3.67, $p<0.0001$), even though all fish had been on the same diets since the beginning of interval two (Fig. 5.3, pg. 69; Fig. 5.4, previous page). This suggests a greater morphological flexibility in offspring of freshwater-residents than anadromous trout.

5.4 Discussion

The diet on which juvenile brown trout were reared had a significant effect on body (especially head) shape and growth, with fish fed on Chironomid larvae having a higher growth rate and developing a more rounded body shape compared to those fed on a *Daphnia* diet. There were initially no differences in SMR between fish on the two diets, nor between fish from different types of parent (i.e. freshwater-resident versus anadromous). However, given that SMR often differs by a factor of 2 or 3 between individual trout fry of the same age and size (Burton et al. 2011), this result may be due to low statistical power to detect differences among groups. Individual differences in SMR within salmon and trout populations have been linked to variation in individual growth and life history strategies (e.g. timing of subsequent smolt migration; McCarthy 2000). Although we did not detect a difference in SMR between offspring type and diet type we did find a decrease in SMR when diets were switched (in either direction). Flexibility in SMR has been shown to occur in salmon and trout populations in relation to food availability, with individual SMR decreasing following a period of food restriction (Du Preez 1987; Wieser et al. 1992) and increasing when food is supplied above baseline levels (O'Connor et al. 2000; Van Leeuwen et al. 2011; 2012); moreover, growth is fastest in those individuals that show the biggest change in SMR in response to changing food availability (Auer et al. 2015). However this doesn't explain the reduction in SMR in our

study as it happened regardless of the direction of the diet switch and despite the fact that the fish were fed an equal ration (in terms of relative mass), calculated to be *ab libitum*, for each diet type. One possible explanation is an imbalance between new prey type, digestive tract performance and assimilation, producing a similar response to when food levels are changed. Vertebrate digestive tracts have been shown to respond over relatively short time scales to differences in prey consumption and food availability (Starck 1999; Armstrong & Bond 2013). For example, snakes can increase the capacity and activity of their digestive tract during a meal, and conversely decrease its capacity and activity during periods of food deprivation (Secor & Diamond 2000). Similarly, juvenile salmonids can dramatically increase the length of their intestine during sustained periods of increased food availability (Armstrong & Bond 2013). The switch in diet may have meant that the digestive system of the fish was initially imperfectly matched to the type of food, which might produce a similar response to a food shortage.

This idea of a difference in digestive requirements for the two food types is supported by the analyses of growth rate. We found a difference in growth rate between diet types and this in turn was affected by the fish's parental type. *Daphnia*, although relatively similar in proximate composition (and hence energy content per unit wet mass) to Chironomid larvae (see methods section above), have a hard exoskeleton; this is likely harder to digest (Swaffar & O'Brien 1996), compared to soft-bodied Chironomid larvae, so it was not surprising that fish grew faster on a diet of Chironomid larvae compared to those fed *Daphnia*. However, offspring of freshwater-resident brown trout were more able to maintain their growth on the *Daphnia* diet than were those of anadromous parents, so that the latter showed a greater fluctuation in growth rate following a switch in diet, indicating potential differences between offspring from the two types of parent in the ability to compensate for changes in diet type. Differences in growth efficiency between freshwater-resident and anadromous individuals have been demonstrated in previous studies. For example, Morinville & Rasmussen (2003) demonstrated that individual migrant brook trout had a lower growth efficiency in the year prior to migration compared to sympatric resident brook trout.

Lastly we found that the extent of the divergence in body shape induced by diet (as measured by Mahalanobis distance between individuals fed Chironomid larvae and *Daphnia*) was similar between offspring types for the first weeks of feeding (i.e. during interval one, the first ~111 days since first feeding, and interval two, the next ~56 days after the diet switch). However, while in offspring of anadromous trout the diet-induced

change in shape was maintained at the same level (as indicated by a relatively constant Mahalanobis distance) for intervals two and three, the offspring of freshwater-resident trout fed on Chironomid larvae continued to diverge in shape over this time period from those fed on *Daphnia*. This suggests a greater plasticity in morphology in the offspring of freshwater-residents. Morphological flexibility in response to diet type is well documented and is generally related to an increase in efficiency of detection, capture and handling of prey items (Skulason & Smith 1995; Adams & Huntingford 2002) and is a primary driver behind the expression of alternative trophic phenotypes. For example Walls et al. (1993) demonstrated that larval eastern long-toed salamanders (*Ambystoma macrodactylum columbianum*) fed tadpoles and brine shrimp nauplii developed significantly broader and deeper heads compared to those only fed brine shrimp nauplii. While we cannot be sure that the morphological differences induced by the two diets in this study were adaptive, the fact that the type of offspring with the greater morphological flexibility (i.e. the offspring of freshwater residents) also showed a greater ability to maintain growth on the poorer prey type is suggestive of an adaptive response. One potential explanation for the difference in morphological flexibility between offspring from alternative life histories is their contrasting requirements for niche shifts. Freshwater ecosystems are often regarded as being food-limited (Imre et al. 2005), so requiring adaptability in diet choice; moreover, freshwater-resident trout tend to move into deeper and slower-flowing habitats as they get older (e.g. deeper pools in rivers, and often eventually lakes; Klemetsen et al. 2003). These ontogenetic changes in diet and habitat likely both require changes to swimming capability and foraging mode (e.g. with the fish becoming less active as they increase in size), so selecting for the ability to remain morphologically flexible throughout ontogeny (to minimise energetic costs and maximise prey capture efficiency). Freshwater-resident individuals may thus benefit from morphological flexibility, since this would help maintain growth in the unproductive and changeable freshwater environment. In contrast, fish migrating to sea will continue to be actively swimming against strong currents and obtaining prey by pursuit foraging, in a highly productive environment that allows narrow dietary specialisations, so possibly selecting against morphological flexibility.

One potential caveat to our study is that we were unable to determine whether the differences between offspring phenotypic flexibility were primarily due to genetic or maternal effects, but this would be difficult to establish given that the resident-anadromous dichotomy by its very nature prevents the use of the standard approach of rearing the parents in a common garden to rule out maternal effects.

In conclusion, the results of this study suggest that offspring from freshwater-resident and anadromous parental life history strategies show some differences in phenotypic flexibility that may be consistent with the future habitats individuals may encounter, with offspring of migratory fish being apparently morphologically less flexible and less able to maintain growth on a poor quality diet. Therefore we suggest that genetic and parental effects affecting phenotypic flexibility may contribute to the differences in performance observed in a common environment and may play a role in the perpetuation of non-breeding partial migration within populations of brown trout.

Chapter Six: General Discussion

The goals of the research presented in this thesis were to investigate the effects of migration history of the parents on offspring development, physiology, behaviour and phenotypic flexibility in salmonid fish. In particular I focused on understanding the differences in fecundity, egg size, developmental rate, condition, growth, SMR, MMR and AS between offspring from freshwater-resident and anadromous brown trout (chapter 2). Using Atlantic salmon, I examined how the duration of early and later life stages of both parents (i.e. their life history) may have a significant influence on offspring traits (chapter 3), and measured the relative dominance of offspring from freshwater-resident and anadromous brown trout when competing for feeding territories and how prior food availability influences these relationships between offspring types (chapter 4). Lastly I examined how offspring from freshwater-resident and anadromous brown trout respond morphologically (in terms of body shape) and physiologically (in terms of SMR and growth) to two diets of differing ease of digestion (chapter 5).

In this chapter I will first recap the current literature and discuss how these results enhance our understanding of partial migration, with reference to the existing literature. I will then discuss the limitations of these studies as a whole, their importance and finally potential future directions of research that arise from it.

6.1 Recap of Current Literature

While variation in the propensity of individuals from a single population to migrate between habitats has fascinated scientists for decades, generalisations about control mechanisms and their adaptive significance remain difficult to quantify (Dodson et al 2013). One reason for this is likely due to differences in the perception of adversity between species and individuals. Adverse environmental conditions are thought to trigger migration (Taylor & Taylor 1977), but a given environmental stressor such as the thermal regime, which may trigger migration in individuals that cannot pay the cost of thermal acclimation (Belthoff & Gauthreaux 1991), may be more important for some taxa (such as many birds) than others (such as fish, which can often tolerate wide thermal ranges). It therefore becomes difficult to quantify the degree to which an environment is truly stressful, and to predict when it should trigger migration in a particular species or individual. From an evolutionary perspective the maintenance of such individual variation in migratory tendency is not much clearer as it encroaches on the understanding of some of

the most fundamental theories of ecology. For example, is the existence of multiple discrete life histories within a population intermediary in the evolutionary process that is moving towards a common life history – or towards the creation of separate species? Or, more plausibly, is the existence of alternative life histories due to the variability of the environment, with the time scale of stochastic events being shorter than the evolutionary time scale that is needed to create a common life history (or separate species)? Despite these limitations in our understanding of partial migration, a common theory among taxa does emerge and is termed the threshold model (Thorpe & Metcalfe 1998; Pulido 2011).

In the threshold model the triggering of migration depends on whether or not a continuous character ("liability trait") exceeds a genetically predetermined threshold value (Chapman et al. 2011; Dodson et al. 2013); given that it has a genetic basis, this threshold value will be to some degree related to (and hence predictable from) the migration history of the parents. In this scenario, at a particular time of year (a time window), individuals physiologically self-evaluate their performance against this threshold (e.g. of growth rate, body size or physiological condition), with migration being dependent on whether or not the threshold is exceeded (Fleming 1996; Thorpe & Metcalfe 1998; Pulido 2011; Dodson et al. 2013).

While the threshold model has gained considerable acceptance amongst the partial migration literature (Fleming 1996; Thorpe & Metcalfe 1998; Pulido 2011; Dodson et al. 2013) there remains a discrepancy as to the directionality of the liability trait in which migration is favoured. For example several studies in fish suggest that it is the faster growing individuals of a population which migrate to more productive environments (Forseth et al 1999; Morinville & Rasmussen 2003), due to these individuals becoming energetically constrained at a younger age and/or smaller body size in low-productivity environments. In contrast, other studies seem to suggest that it is the slower growing individuals of a population which are more likely to migrate, with faster growing individuals remaining resident and reaching sexual maturity at a younger age and smaller body size (Olsson et al. 2006). This discrepancy, along with earlier work by Metcalfe (1998) using Atlantic salmon, has led to the conclusion that migration may actually occur at both extremes of a liability trait (Paez et al. 2011; Rossignol et al. 2010). Metcalfe (1998) demonstrated that the juvenile Atlantic salmon growing fastest prior to the winter of their first year of life can become early migrants the following spring, whereas slower

growing individuals become either resident (as male precocious parr) or delayed migrants which migrate the following year.

While these models generate variation in migratory tendency between environments they can also generate partial migratory populations even under uniform conditions (since the threshold can vary between individuals and species depending on their inherent susceptibility to migrate); however, hard evidence of the factors that pre-dispose particular individuals to one or the other strategy remain lacking.

6.2 Furthering Our Understanding

While the decision to migrate is undeniably influenced by both genetic and environmental components, the results of this thesis suggest that parental effects (either direct or environmentally-mediated through differences in the environment they experienced earlier in life) may be an important mechanism underlying the expression of threshold traits; these parental effects appear to play a significant role in the perpetuation of non-breeding partial migration and life history strategies within populations of brown trout and Atlantic salmon.

I found that freshwater-resident parents produced eggs that were slower to hatch than did anadromous parents, but their offspring were quicker to absorb their yolk and reach the stage of exogenous feeding. Their offspring also had a higher conversion efficiency from the egg stage to the start of exogenous feeding (so were larger by the start of the fry stage) and demonstrated greater morphological flexibility at the fry stage (chapter 5) than did offspring from anadromous parents. These offspring however were competitively dominant over size-matched offspring of freshwater-residents, but only when both had been raised under intermediate levels of food availability.

Although anadromous offspring were competitively dominant in size-matched trials (chapter 4) the importance of this result in the context of the earlier findings of chapter 2 (offspring of freshwater-residents being of a large size at the start of exogenous feeding) remains unclear. Given that freshwater-resident offspring were larger at the start of the fry stage (chapter 2), it is likely that these individuals would have a greater competitive ability (Einum & Fleming 1999) since a larger body size (Cutts et al.1999) generally allows for preferential access to food, shelter or mates, regardless of a potentially higher inherent competitive ability.

Interestingly, anadromous offspring were only competitively dominant over size-matched freshwater-resident offspring when both categories of offspring had been raised on intermediate levels of food availability. This asymmetry suggests that the two parental types differ in the environmental threshold at which they adopt a more competitive and aggressive foraging strategy, which could have been related to the timing of the experiment with respect to whether or not individuals were destined to migrate. Given their size at the time of the experiment, the earliest that any of the experimental fish would migrate to sea would be in the following spring. Morinville & Rasmussen (2003) demonstrated that individual migrant brook trout had higher food consumption rates and lower growth efficiencies in the year leading up to migration compared to sympatric resident brook trout, suggesting that the migrant brook trout had higher metabolic costs (although we did not find any differences in our study population of fish in SMR, MMR and AS; chapter 2). Furthermore, Thorpe et al. (1998) proposed that the decision to migrate in the closely-related Atlantic salmon occurs soon after mid-summer (around the time of this study): under this model, fish that fall below their individual threshold for growth rate at this time subsequently decline in appetite, cease growth and defer migration, while those individuals who are above the growth threshold maintain their appetite throughout late summer and autumn and undergo migration the following spring. If this pattern of migration holds for brown trout, then all fish in the low food treatment may have already failed to reach the threshold triggering migration, and so would be programmed to have a declining appetite and hence reduced need to compete for food. However, if the tendency to migrate is partially genetically determined (Berthold 1988; Berthold & Pulido 1994), then under the mid food treatment offspring of migrants might have had a lower growth threshold triggering migration, so would have a greater motivation to acquire feeding territories so as to be able to maintain their growth rate. Meanwhile all fish reared on the high food treatment may have passed their respective thresholds, leading to aggressive and dominant behaviour being expressed by both parental types in preparation for migration the following spring, and hence no consistent difference in dominance status between them.

While my results demonstrate that parental effects appear to play a significant role in the perpetuation of non-breeding partial migration and life history strategies within populations of brown trout (chapters 2,4,5) and Atlantic salmon (chapter 3), it remains unclear if these differences have a genetic basis (i.e. due to genetic differences), or are parental effects, arising as a by-product of differing environments experienced by the two

types of parents prior to spawning. The results of our Atlantic salmon experiment (chapter 3) suggests the latter as this study suggested that not only does the environment that parents inhabit influence offspring traits but so does the duration of early and later life stages of both parents (i.e. their life history); with the traits affected being ones that are likely to influence the age of offspring migration. Mothers that had spent longer at sea prior to spawning (two versus one year) produced offspring which were heavier, longer and in better condition at the time of first feeding. However, these relationships disappeared shortly after fry had begun feeding exogenously. At this stage, the early rearing environment (i.e. time spent in fresh water) of the mother was a better predictor of offspring traits, with mothers that were faster to develop in fresh water (migrating to sea after two rather than three years of age) producing offspring that had higher MMR and AS, and that grew faster. Faster developing fathers tended to produce offspring that grew faster, were in better body condition and had higher MMR; these paternal effects are likely to have a genetic basis since the father contributes nothing more than sperm in this species (so making non-genetic paternal effects unlikely), whereas the maternal influences could be through genetic or non-genetic means (e.g. through differential provisioning of the egg).

Although my study design did not allow me to test the relative contributions of the mother and father in the brown trout studies, the results of the Atlantic salmon study (chapter 3) suggest that both sexes could influence offspring traits in brown trout, including via maternal effects driven by the earlier environment experienced by the mother. Although the brown trout has less tendency to migrate than the Atlantic salmon, there is still reason to expect it to show environment-dependent variation in maternal provisioning of the egg (and subsequent development of the offspring). Mothers living in low food environments often exhibit reduced growth, smaller adult size and lower lipid reserves, and in turn tend to produce fewer but larger eggs (Taborsky 2006), whereas those in high food environments show greater growth, larger size and higher lipid reserves and tend to produce a greater number of smaller sized eggs (Burton et al. 2013). Furthermore Braun et al.(2013) demonstrated that in years when migration was challenged by high water discharges, female Sockeye salmon invest less in gonadal development and produce smaller but not fewer eggs, consistent with other studies demonstrating a negative relationship between egg size and migration cost (Fleming & Gross 1990; Kinnison et al. 2001). Therefore, given both the likelihood of non-breeding partial migration to generate variation in the pre-breeding environment (Burton & Metcalfe 2014) and the further constraints due to migration, it follows that anadromous females should be producing a

greater amount of smaller eggs compared to freshwater-resident females. Furthermore the results of my studies also suggest that these environments also shape later developmental stages but in a way that is likely to increase the probability of offspring following in their parents' trajectory with freshwater-resident parents producing offspring that are quicker to reach the stage of exogenous feeding, having higher conversion efficiency and greater morphological flexibility compared to offspring from anadromous parents.

6.3 Limitations of My Studies

While my results are robust for my populations of trout and salmon I cannot ignore the lack of replication at the population level in my studies, therefore it is possible that the patterns that I found may not hold true across other populations, although this requires further examination. Replication at the population level, while still controlling for between-family variation (as I did in my studies), is logistically very difficult when conducting common-garden studies on salmonids. This is due to the large numbers of eggs and offspring produced per female, which makes housing and husbandry difficult due to the vast amounts of offspring accumulated when accounting for population- as well as ecotype- and family-level effects. Therefore, many partial migration studies on salmonids and other taxa have involved the use of only single populations (Chapman et al. 2011; Reznick & Endler 1982; Olsson et al. 2006). This is especially true of studies of wild populations, due to the logistical constraints of measuring and following multiple individuals across contrasting environments and sometimes vast geographical ranges. Therefore in this thesis I make the assumption that between-family variation (which was relatively controlled for in my studies both statistically, by controlling for non-independence of siblings and experimentally, by mating several males and females) is likely to be higher than the between-population variation (which is not controlled for in my studies).

Another limitation with my brown trout studies is that I was unable to compare the offspring from crosses of freshwater-resident X anadromous brown trout, which did not allow for the relative contribution of the mother and father to be examined in the brown trout studies (chapters 2,4,5). It is often presumed that the mother's contribution (in terms of either the overall amount of nutrients, or specific modifiers such as maternal hormones, that are deposited in the eggs) is likely to be the most influential on offspring traits (Bety et al. 2003; Jonsson & Jonsson 2014), but there is some evidence from my study involving Atlantic salmon (chapter 3) that the father may also have an influence. Although not tested here it would be interesting to repeat a similar experimental design as in my Atlantic

salmon experiment (chapter 3) to better understand the contribution of migration history of the parents to the probability of offspring migration.

6.4 Importance of Understanding Partial Migration

Understanding variation in the propensity of individuals from a single population to migrate between habitats is fundamental at multiple scales since it can shape the destiny of individuals and populations, and hence ecosystem structure and function (Polis et al. 2004) as these species form a conveyor to which productivity and nutrients can be transported from one ecosystem to another (Limburg & Waldman 2009). In light of this ecological role, it is important that we gain an understanding of how these migratory populations are likely to evolve in light of the increasing challenges of habitat destruction and climate change (Wilcove 2008; Chapman et al. 2011). One possible outcome of climate change and anthropogenic disruptions to ecosystems could be shifts in the proportions of individuals which undergo migration. For example Nilsson et al. (2006) demonstrated an increase in the proportion of migrant blue tits in response to climatic warming, although the opposite situation is as equally to occur depending on location, species and degree to which adversity is shifted.

Another possibility is that partially migratory species may be more resilient (in terms of extinction risk) to climate change and anthropogenic disruptions compared to fully migratory species (Chapman et al. 2011). For example the ability of a single species to exist across multiple habitats may allow one ecotype to act as a buffer for the species (source population) against extinction compared to more fully migratory species which are likely to have a narrower breadth of tolerance (by sheer nature of being fully migratory, provided that migration is a response to adversity), although this has yet to be evaluated.

While understanding how partially migratory populations will respond to climate change and anthropogenic disruptions is important, an additional problem is the ability to which these species can be effectively managed. For example, the geographical expanse over which migratory ecotypes are likely to traverse may require border-wide jurisdiction and enforcement to be effective, regardless of whether the species has protection at either ends of the migration. A further complication is that in some species one ecotype may be more vulnerable or sought after by hunters and fishers than the other ecotype. For example, precocious male parr in Atlantic salmon and freshwater-resident brown trout are not as highly regarded by anglers as their anadromous counterparts, leading to different

regulations and management practices being applied to the different ecotypes of the same species.

Populations of both the Atlantic salmon and the brown trout (especially the anadromous form) have reduced markedly in recent decades, with some populations declining by as much as 80% (Limburg & Waldman, 2009). Despite these dramatic declines the two species continue to be an important economic and social asset to both recreational and commercial fisheries. Reasons for the population reductions in these two study species remain unclear but anthropogenic disturbances (in-stream barriers, habitat degradation, over fishing, fish farming) and climate change have been identified as the most likely contributors (Limburg & Waldman 2009). Anadromous species of fish are thought to be particularly vulnerable to these effects and more at risk of population extirpations because they generally contain numerous, smaller, locally adapted populations (Chapman et al. 2011; Eliason et al. 2011) compared to relatively few but large geographically widespread populations of non-anadromous species (Limburg & Waldman 2009).

6.5 Potential Future Directions for Research

Despite the wealth of studies on partial migration there continues to be several avenues that warrant further study. Further studies should involve long-term common garden experiments which expand over several populations and involve the use of other species. By rearing full-sibling or half sibling crosses on differing food regimes over the long-term (or at least until migration is initiated) the relative contributions of environment and genetics to partial migration could be investigated. Furthermore by using a factorial approach in which 'hybrids' of the two migratory types are reared in a common garden, the relative contributions of the mother and father to partial migration could be examined separately.

Future field studies could involve the use of telemetry to help track the movements of individuals across habitats, so allowing for the quantification of the proportion of individuals within populations that adopt alternative life histories. While constantly evolving, tagging technology can be used on an increasing number of species and can range from acoustic tags which could be beneficial in tracking the movements of fishes to global positioning tags that can be used to track movements of terrestrial species.

Lastly, genomics could be used to investigate the genetic bases of alternative life history strategies which may allow for the relative genetic contribution to partial migration to be investigated.

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Appendix

Chapter A-1: Does Individual Variation in the Metabolic Phenotype Predict Behaviour and Performance in Fish

*Note: This chapter was co-written with Prof. Neil Metcalfe and Dr. Shaun Killen and has been accepted for publication as a review paper for a special issue of the Journal of Fish Biology on fish physiology. For this manuscript I was responsible for the sections entitled "Individual variation in metabolic responses to feeding and food availability" and "Individual variation in metabolic responses to abiotic factors".

A.1 Introduction

Fishes have provided some of the best examples of intraspecific variation in morphological features that reveal adaptations to local environments. These include differences in gill raker spacing and mouth shape as adaptations to feeding on benthic vs. planktonic resources (Schluter, 1993), changes in body shape that reduce the risk of predator attack (Brönmark & Miner, 1992) or increase the ability to escape (Walker, 1997), and variation in shape related to habitat differences in water velocity (Peres-Neto & Magnan, 2004). Recently however, it has become clear that more cryptic physiological measures may be equally important in explaining patterns of adaptation and life history diversity within species. The increasing interest in the role of physiology in shaping evolution (Irschick & Garland, 2001; Irschick *et al.*, 2008) and in mediating life history trade-offs (Zera & Harshman, 2001; Monaghan *et al.*, 2009) has focussed attention on individual variation in physiological traits. Such traits have historically been more poorly documented than morphological features. However, recent technological and analytical advances (e.g. the development of oxygen sensing software and technology ('optodes') for respirometry of aquatic organisms – see Nelson (Nelson, 2014 - this issue) have made it increasingly possible to obtain data from sufficient numbers of individuals to quantify the level of intraspecific variation in a range of physiological traits. This has led to a relatively new approach to ecophysiology which is focused on understanding the causes and ecological consequences of individual variability in physiological traits, rather than treating it as 'noise' or 'error' between measurements.

One of the main physiological traits of interest in this study of intraspecific variation has been metabolic rate – partly because it can be measured using non-invasive means and partly because it is assumed to have broad ecological relevance (Burton *et al.*, 2011b). Standard metabolic rate (SMR), which is equivalent to basal metabolic rate (BMR) in endotherms, is the minimal maintenance metabolic rate of an ectotherm in a post-absorptive and inactive state (Chabot *et al.*, 2014b - this issue). SMR (usually measured in

terms of oxygen consumption) is an integrated measure of the physiological energy expenditures involved in the anabolism and catabolism of tissues and organism homeostasis. After controlling for temperature and body size, SMR varies among fish species in relation to their lifestyle (e.g. benthic vs. pelagic) (Killen *et al.*, 2010). However, after controlling for these sources of variation, SMR often differs by a factor of up to 2 or 3 among individual fish of the same age, sex and species held in similar conditions (Millidine *et al.*, 2009; Norin & Malte, 2011; Killen *et al.*, 2012b). The related term routine metabolic rate (RMR) refers to the average rate of metabolism when the animal is undergoing normal behaviours or some other specified type of activity (although these should be carefully defined). The term resting metabolic rate, though somewhat vague and not ideal when applied to fishes, is sometimes used when interchangeably discussing both SMR and RMR. Aerobic scope (AS) is defined as the difference between an animal's SMR and its maximum possible aerobic metabolic rate (MMR) under the same environmental conditions, so that aerobic scope defines the capacity of the animal to increase its rate of aerobic metabolism (Norin & Clark, 2014- this issue). Together, these metabolic traits make up what will be referred to as the metabolic phenotype of the individual. The focus will mostly be on SMR, MMR and AS since their meaning is more precisely defined. While there have been fewer studies of MMR and AS than there have been of SMR, these two related measures of metabolism show similar levels of intraspecific variation (Norin & Malte, 2011; 2012; Killen *et al.*, 2012b).

The extent of this intraspecific variation in metabolic rate is in line with that found in other taxa (Steyermark *et al.*, 2005; Johnston *et al.*, 2007). Given the profound consequences that this has for energy budgets, its persistence demands explanation and has been the subject of a number of recent reviews that attempt to link intraspecific variation in minimal or maximal metabolism to other biological traits or ecological factors (Gomes *et al.*, 2004; Biro & Stamps, 2010; McKechnie & Swanson, 2010; Burton *et al.*, 2011b; Konarzewski & Ksiazek, 2013). Here we aim to provide a synopsis on the current understanding of intraspecific variation in these various components of the metabolic phenotype and their links to other traits in fishes, by specifically focusing on i) the extent and causes of individual variation and repeatability of metabolic traits; ii) how the metabolic phenotype varies with environmental conditions (food availability, temperature, hypoxia, salinity and shelter); and iii) the relationships between the metabolic phenotype and other whole-organism traits (such as dominance, aggression, risk-taking, boldness and swimming performance). We then conclude by outlining several key areas where we feel that information is currently lacking. Our goal is to understand how this individual

variation in metabolism arises and persists by considering its consequences under different ecological conditions.

A.1.2 Individual Variation in Metabolism

A.1.2.1 The Consistency of Individual Variation in Metabolic Rate

If measurements of metabolic rate are taken at only a single point in time (as is the case in the great majority of studies) then there is the risk that the observed variation between individuals is simply due to measurement error or random temporal fluctuations in metabolism. However, there have now been enough studies that have taken repeated measurements from the same individuals to allow a proper evaluation of the consistency of these traits over time. The initial studies showed that individual differences in SMR tended to be repeatable: measures of relative SMR (i.e. after correcting for differences in body mass) in the same fish but at different times were found to be significantly correlated, with correlation coefficients ranging from 0.40 (O'Connor *et al.*, 2000) to 0.68 in Atlantic salmon *Salmo salar* L. 1758 (McCarthy, 2000). Significant repeatabilities were also found in individual Arctic charr *Salvelinus alpinus* L. 1758 measured at intervals of up to 6 months (Cutts *et al.*, 2001).

This body of work, together with a meta-analysis of metabolic rates across the vertebrates (Nespolo & Franco, 2007), led to the general consensus that minimal rates of metabolism were stable traits of individuals. This has been supported by more recent studies of other fish species (e.g. correlation coefficients of 0.68-0.73 in Spined loach *Cobitis taenia* L. 1758 (Maciak & Konarzewski, 2010), 0.71 in Arctic charr (Voutilainen *et al.*, 2011) and 0.50 in European eel *Anguilla anguilla* L. 1758 (Boldsen *et al.*, 2013). However, this view has recently been challenged by new evidence suggesting that the repeatability decreases over time (Norin & Malte, 2011; White *et al.*, 2013). The highest correlations between repeated assessments of an individual's SMR occur when the time interval between the two measurements is short (the most extreme case being r=0.88 for Bleak *Alburnus alburnus* L. 1758 when the two respirometry trials were separated by only an hour (Voutilainen *et al.*, 2011)). As the interval between trials increases, the measurements of relative SMR become less similar. The most detailed analysis of this effect comes from the study of Brown trout *Salmo trutta* L. 1758 by Norin & Malte (2011), who demonstrated a decline in correlation coefficients from 0.57 to 0.09 as the time interval between measurements increased from 35 to 105 days. The rate at which the repeatability decreases is not constant: relative SMR was found to remain very consistent (r = 0.68) over 17 weeks in juvenile *S. salar* (despite a 20-fold average increase in their

body size)(McCarthy, 2000). In contrast, slightly older individuals of the same species were found to exhibit no repeatability in relative SMR when measurements were separated by 6 months (r = 0.02) or 14 months (r = -0.17), although there was a significant correlation at an interval of 8 months (r = 0.51) (Seppänen *et al.*, 2010). It is worth noting, however, that the only studies examining temporal changes in repeatability in fishes have been conducted on populations held under homogenous laboratory conditions. It is therefore possible that in this situation behavioural feedbacks associated with food acquisition, habitat choice, or dominance hierarchies, all of which may affect the metabolic rate of individuals, are largely prevented from exerting effects that could otherwise preserve repeatability over longer temporal scales. More work is therefore needed to examine the repeatability of metabolic traits in semi-natural or natural conditions.

Changes in environmental conditions or the physiological state of the fish may also act to erode repeatability in metabolic rate: no correlation was found between the relative SMR of juvenile *S. salar* when measured on *ad libitum* rations and when measured after having been deprived of food for 3 weeks, although the correlation was restored (r=0.40 between initial and final measurement) once the fish had been back on *ad libitum* food for 4 weeks (O'Connor *et al.*, 2000). Similarly the correlations between repeated measurements of relative SMR in Barramundi *Lates calcarifer* Bloch 1790 were weaker when comparing tests carried out in contrasting environments (e.g. differing temperatures, salinities or levels of hypoxia) compared to the same environment (Norin, 2014).

There have been far fewer studies of the within-individual repeatability of MMR or AS, but to date these show the same trends as for SMR. Thus studies of *S. trutta*, *A. alburnus* and *S. alpinus* suggest that there is a significant repeatability in MMR and AS that tends to decline with increasing time intervals between measurements, at least in the simplified environment of the laboratory (Norin & Malte, 2011; Voutilainen *et al.*, 2011), and also declines with a change in environmental conditions (Norin, 2014).

A.1.2.2 The Underlying Causes of Individual Variation in Metabolism

There has been speculation over the physiological differences that generate intraspecific variation in metabolic rate, but even when considering other taxa the picture is not clear, whether the metabolism is minimal (Burton *et al.*, 2011b; Konarzewski & Ksiazek, 2013) or maximal (Gebczynski & Konarzewski, 2009). A number of studies (reviewed by Konarzewski & Ksiazek, 2013) have shown that both interspecific and intraspecific variation in body-size corrected BMR is positively associated with variation in the relative size of metabolically-expensive organs such as the heart, liver and brain. However,

relationships between organ size and metabolic rate appear to be less evident in fishes, possibly because the key organs make up a smaller proportion of the total body size in fishes compared to endotherms. While relative liver size was found to be a predictor of relative SMR in *A. anguilla* (Boldsen *et al.*, 2013), neither SMR nor MMR were related to the sizes of any of the key organs in *S. trutta* (Norin & Malte, 2012). Instead, intraspecific variation in both SMR and (to a lesser extent) MMR in the latter species were positively correlated with the activity of two key aerobic mitochondrial enzymes (cytochrome C oxidase and citrate synthase) (Norin & Malte, 2012). Negative relationships between SMR and erythrocyte size have also been found in individual *C. taenia* differing in ploidy levels (Maciak *et al.*, 2011), but while variation in cell size may explain some of the interspecific variation in metabolic rate, the uniformity of cell size in most species makes it unlikely that this is a major contributor to variation in metabolic rate within species.

Less is known about the factors influencing MMR. Cross-species analyses suggest that MMR is constrained by the mechanics of both oxygen and carbon dioxide transport (Hillman *et al.*, 2013). Several studies have documented correlations among SMR, MMR, and AS across individuals. The existence of these associations suggests a functional link among these traits, but the direction of the correlations vary among species. Positive correlations have been observed between SMR and MMR for grass carp *Ctenopharyngodon idella* (Cuvier & Valenciennes 1844) (Zhang *et al.*, 2014) and *S. trutta* (Norin & Malte, 2012), and between resting metabolic rate and MMR following feeding in southern catfish *Silurus meridionalis* (Chen 1977) (Fu *et al.*, 2005). In contrast, SMR and measures of AS are negatively correlated in the Common minnow *Phoxinus phoxinus* L. 1758 (Killen, 2014) and juvenile *S. salar* (Cutts *et al.*, 2002). The nature of the relationships among these traits may depend on the species in question and the exact metabolic indices measured. Further, it is possible that the pattern of energy budgeting and allocation within species can affect correlations among traits within individuals.

A separate question is whether these differences are primarily under genetic or environmental control. While there are very few studies of the genetics of metabolic rate in fishes, mass-independent BMR has been shown to be heritable in birds (Mathot *et al.*, 2013) and mammals (Zub *et al.*, 2012; Boratynski *et al.*, 2013), as has MMR (Wone *et al.*, 2009), and there is evidence of a paternal effect on SMR in *S. salar* (Pakkasmaa *et al.*, 2006). SMR has also been found to differ between individuals from different populations of *S. salar* and *S. trutta*, even when reared in a common environment (Lahti *et al.*, 2002;

Seppänen *et al.*, 2009b; Seppänen *et al.*, 2009a), which again suggests a genetic component to intraspecific variation in metabolic rate in fishes.

However, it is also clear that metabolism can be influenced by maternal (i.e. non-genetic) effects (Régnier *et al.*, 2010): the routine metabolic rate of *S. salar* fry and the relative SMR of *S. trutta* embryos were both found to decrease with increasing egg size (Rossignol *et al.*, 2010; Régnier *et al.*, 2012), while Burton *et al.* (2013) found SMR in *S. trutta* fry to vary according to the position that the egg occupied in the mother's ovary (with stronger positional effects in more dominant mothers). The reason for these effects is not known, but may relate to variation in the composition of the eggs: female fish deposit hormones into their eggs, and manipulations of egg cortisol levels have been found to influence the SMR of the resulting offspring in *S. trutta* (Sloman, 2010) (although note that a slightly different method of manipulating cortisol levels in *S. trutta* eggs found no such effect (Burton *et al.*, 2011a)).

A.1.2.3 Individual Variation in Metabolic Responses to Feeding and Food Availability

Specific dynamic action (SDA), the increase in organismal metabolism as a result of food ingestion (Chabot *et al.*, 2014a - this issue), can constitute upwards of 60–80 % of the maximum rate of oxygen consumption (Alsop & Wood, 1997) or 2-3 times the baseline metabolic expenditure and in fishes can last anything from 3 to 390 hours (Secor, 2009). In some sedentary species the peak in oxygen uptake following feeding can exceed that observed during peak aerobic exercise (Fu *et al.*, 2005). Although much of the variation in duration and peak SDA can be explained by differences in fish size and species, ambient temperature, and composition of a given meal (Secor, 2009), there remains considerable variation that can likely be attributed to inherent differences between individuals. For example, in both *S. meridionalis* and *S. salar* it has been shown that individuals with a relatively high SMR for their size had a higher peak and overall magnitude of SDA but had a shorter duration of the SDA response, indicating that the metabolism of these individuals was quicker to return to resting levels after each meal compared with low SMR individuals (Fu *et al.*, 2005; Millidine et al. 2009). Based on these results it was thought that individuals with a high SMR and consequently a shorter SDA response may have a growth advantage under high food conditions, since a strategy based on quick ingestion and assimilation may allow for greater throughput of food and ultimately growth; high SMR fish may also be more dominant (see below) and so have priority of access to food. Under conditions of low food availability, however, this strategy may become maladaptive due to

the higher energy demands placed on individuals with a high SMR, which is not offset by any increased food intake. It should be noted that the rate of growth may itself influence the estimate of SMR, depending on the protocol that is used (Rosenfeld *et al.*, 2014), which may help explain inconsistences found between studies that relate individual differences in metabolic rate to differences in growth rate (Hoogenboom *et al.*, 2013). Laboratory studies (in which food is generally supplied *ad libitum*) have generally found a positive correlation between SMR and growth rate (Cutts *et al.*, 1998; Yamamoto *et al.*, 1998), whereas equivalent analyses carried out on fishes in conditions where food is more likely to be limiting (e.g. the natural environment) have found either negative or no correlations (Álvarez & Nicieza, 2005; Finstad *et al.*, 2007b; Norin & Malte, 2011; Robertsen *et al.*, 2014). It has been suggested that the fitness advantages of a given SMR may thus depend upon the context (Burton *et al.*, 2011b), a hypothesis that is supported by recent stream tank experiments showing that SMR is positively correlated with growth rate when fishes are competing for access to a plentiful food supply but the relationship becomes negative once access to food is taken into account (Reid *et al.*, 2011; 2012).

While the direct energetic consequences of SDA are not included as part of SMR, MMR or AS (and are controlled for by fasting individuals prior to measurement), the indirect effects of the metabolically expensive organs associated with shaping the SDA response may play a pivotal role in explaining individual differences in metabolism (Rosenfeld *et al.*, 2014) . However, what remains inconsistent is whether the size of metabolically expensive organs associated with ingestion, digestion and assimilation (i.e. stomach, liver, heart and intestine) drives the individual differences in baseline metabolism. For example, as mentioned earlier, Norin and colleagues found no relationship between variation in metabolism (SMR, MMR and AS) and mass of metabolically active organs in *S. trutta* (Norin & Malte, 2012) but did find a positive relationship in *A. anguilla*, with 38% of the variation in SMR being explained by the mass of internal organs, in particular the liver (Boldsen *et al.*, 2013). Whatever the driver of this relationship it is clear that individual differences exist in many aspects of the SDA response (e.g. time to response, peak and duration; Millidine *et al.*, 2009), which will likely have implications for individual variability in the metabolic phenotype irrespective of the routinely applied protocol of fasting fish prior to respirometry.

Although the SDA response is the most recognizable and documented effect of food on metabolism, the role of food availability in influencing individual variation in

metabolic rate independent of SDA should not be ignored. There are numerous studies documenting a decrease in SMR when fishes are subjected to a period of food restriction (Beamish, 1964; Du Preez, 1987; Wieser *et al.*, 1992) and an increase in SMR when food is supplied above baseline levels (O'Connor *et al.*, 2000; Van Leeuwen *et al.*, 2011; 2012). While there have been few studies investigating the effect of food level on MMR and AS, those that exist suggest that food level has contrasting effects on MMR and AS. Van Leeuwen *et al.* (2011) found no relationship between food level and MMR in juvenile Steelhead *Oncorhynchus mykiss* (Walbaum 1792) or Coho salmon *Oncorhynchus kisutch* (Walbaum 1792) but did find a negative relationship between food availability and AS. In addition, *P. phoxinus* that experienced a period of food deprivation and subsequent compensatory growth were observed to have an elevated SMR and reduced AS compared to fish that had been feeding ad libitum throughout the entire period, although there was no change in MMR (Killen, 2014).These results suggest that an individual's MMR may be relatively uninfluenced by the fish's nutritional state and that AS is primarily driven by the degree of plasticity within an individual's SMR.

What remains unclear is what processes are responsible for the changes in SMR with varying food conditions. SMR is the culmination of a large number of background processes that consume oxygen (Darveau *et al.*, 2002), and so the variation in SMR due to nutrition could be due to changes in cell maintenance, cell growth and organ mass, but there may also be changes in an individual's investment in energetically costly tissues related to its ability to obtain food (e.g. its lifestyle, level of locomotor performance and competitive ability). This warrants further investigation, but it is clear that an individual's nutritional condition must be taken into account when undertaking measurements of SMR and AS.

A.1.3 Individual Variation in Metabolic Responses to Abiotic Factors

Relationships between metabolic rate and temperature have formed the basis for some of the most fundamental and debated general theories in ecology, ranging from the metabolic theory of ecology (Gillooly *et al.*, 2001) to the more recently proposed metabolic level boundaries hypothesis (Glazier, 2010), with incorporation of an organism's lifestyle (Killen, Atkinson & Glazier, 2010), and the oxygen and capacity limited thermal tolerance (OCLTT) hypothesis (Pörtner & Knust, 2007; Clark *et al.*, 2014 - this issue). While the effects of temperature on the metabolism of fishes have been well documented (Fry, 1971), the great majority of studies have ignored individual variation in that response, the available evidence suggests that not all members of a population have the same

reaction norm: an individual's relative SMR, MMR or AS at one temperature is therefore not a precise predictor of its metabolism if the temperature changes (Norin, 2014).

As with temperature, the metabolic response to hypoxia is generally context-dependent (Claireaux & Steffensen, 2014 - this issue). MMR and AS are likely to be more affected by hypoxia than is SMR, given that depleted oxygen concentrations are more likely to impact the maximum performance of the cardiovascular system than baseline metabolism. Dupont-Prinet *et al*. (2013) found that acute hypoxia did not affect SMR in juvenile Greenland halibut *Reinhardtius hippoglossoides* Walbaum 1792 but did significantly reduce MMR and AS, consistent with other studies across fishes (Petersen & Gamperl, 2010; Norin, 2014). However, given the fundamental role of metabolism in mediating the response to hypoxia and the variability in individual metabolism, it is likely that individuals with a higher or lower metabolic rate will respond differently (both physiologically and behaviourally) to hypoxia exposure. Norin (2014) found that MMR and AS decreased under hypoxia, but that low MMR and AS individuals were much less affected than their high MMR and AS counterparts. These results suggest that individuals with a higher aerobic capacity (MMR, AS) are more likely to be constrained by decreases in oxygen compared to low metabolic rate individuals. Therefore hypoxia may play a significant role in shaping individual personality, although the ecological consequences of these results in the wild are relatively unknown. Results from a laboratory study conducted by Killen *et al*. (2012a) found a positive correlation between SMR and activity, risk taking and aquatic surface respiration (ASR) in European sea bass *Dicentrarchus labrax* L. 1758, but only under conditions of severe hypoxia. The results suggested that as oxygen levels in water decline, high SMR individuals are the first to be forced to expose themselves to greater predation risk to counteract the decrease in oxygen concentration (Killen *et al.*, 2012a). This study supports the work of Domenici, Lefrancois & Shingles (2007) who suggested that a trade-off may exist between fish undergoing ASR to avoid the detrimental effects of hypoxia and vulnerability to aerial predation.

The movement of osmotic solutes across the osmotic gradient is generally regarded as being energetically "expensive", and in extreme cases may constitute upwards of 20-50% of the total energy budget in fishes (Boeuf & Payan, 2001). Fishes in freshwater environments tend to have a lower energetic cost associated with osmoregulation (as revealed by a lower SMR) compared with those in full-strength saltwater (Morgan & Iwama, 1991; Kitano *et al.*, 2010), although other studies comparing sticklebacks from populations experiencing differing osmoregulatory demands have found differences in

MMR and AS but not SMR (Dalziel *et al.*, 2012). Given the degree of interspecific variability in salinity tolerance it is likely that some intraspecific variation may exist, although studies have been scarce. Norin (2014) found that individual *L. calcarifer* with a relatively low SMR, MMR and AS showed an increase in their SMR, MMR and AS when salinities were decreased from 35 ppt to 10ppt, whereas fish with relatively high metabolic rates showed a reduction in their SMR and no real change in MMR and AS when exposed to the same decrease in salinity, indicating that changes in salinity will not have the same consequences for metabolic performance in all members of a population.

It is well established that shelter plays a fundamental role in the survival of animals. Not only does it provide a safe refuge from predators but it also provides a refuge from the environment (Millidine *et al.*, 2006). While the benefits of shelter for fish growth performance is well established (Finstad *et al.*, 2007a), recent evidence suggests that it may also influence the physiology of fishes, even in the absence of predators. The presence of shelter may cause a decrease in the metabolic rate of *S. salar* (Finstad *et al.*, 2004; Millidine *et al.*, 2006) and Burbot *Lota lota* L. 1758 (Fischer, 2000), although no such relationship was found in the false clownfish *Amphiprion ocellaris* Taylor 1882 (Kegler *et al.*, 2013) or the Stone loach *Barbatula barbatula* L. 1758 (Fischer, 2000), indicating that the relationship between shelter and metabolism may be species dependent. It is noteworthy that although the studies demonstrating a reduction in metabolic rate used very different types of shelter (ranging from clear semi-circular Perspex (Millidine *et al.*, 2006) through cobbles (Fischer, 2000) to ice cover (Finstad *et al.*, 2004)), the reduction in metabolism was very similar, being approximately 30% in the presence of shelter. While the reasons for the observed relationship between shelter and metabolism are unknown it is thought that the presence of shelter may reduce stress levels or decrease vigilance (a heightened state of body awareness in response to predation) which may in turn elevate opercular ventilation and ultimately metabolism (Millidine *et al.*, 2006).

Given that metabolism has been found to vary considerably among individuals and influence key behavioural traits that will likely determine shelter use such as boldness, dominance and aggression (Metcalfe *et al.*, 1995; Cutts *et al.*, 1998; Killen *et al.*, 2011), it is likely that the metabolic response of individuals to shelter will vary accordingly to their own baseline metabolic rate. One possibility is that individuals with a low SMR will show a larger increase in metabolism in the absence of shelter compared with those with a higher SMR, given that the latter fish tend to be more aggressive and bold (see below). Although

the presence of shelter will not likely impact MMR, a decrease in AS due to the elevation of SMR in the absence of shelter is a possibility.

A.1.4 Links between the Metabolic Phenotype and Behaviour

A.1.4.1 Aggression and Dominance

To better understand the ecological relevance of intraspecific variation in metabolic traits, a large research effort has been devoted toward examining the associations between metabolic traits and the behaviour of individual animals (Table A.1). Of the potential behavioural correlates of metabolic traits in fishes, aggression and dominance have received the most attention. A general observation is that individuals with higher standard or resting metabolic rates are more aggressive and more likely to become dominant over conspecifics (Metcalfe *et al.*, 1995; Cutts *et al.*, 1998; Yamamoto *et al.*, 1998; Cutts *et al.*, 1999; McCarthy, 2001; Sloat & Reeves, 2014), though exceptions have been noted (Killen *et al.*, 2014). Positive correlations between aggression and SMR among populations of the same species have also been observed (Lahti *et al.*, 2002), possibly driven by local variation in factors such as food availability. In contrast, Seppänen *et al.* (2009b) observed variation in SMR among populations of juvenile *S. salar* that were not associated with differences in levels of aggression.

The causal direction of the positive association between aggression and metabolic rate is unclear. Intrinsic differences in metabolic rate may motivate some individuals to be more aggressive to obtain food or territory; conversely, in cases where metabolism has been measured during or shortly after physical interactions, the measurement could be influenced by the activity costs of aggressive behaviours (Neat *et al.*, 1998; Killen *et al.*, 2014). Several studies of cichlid species have found positive relationships between the rate of aggression (whether lateral displays, fin beats or actual attacks) and metabolic rate (Grantner & Taborsky, 1998; Ros *et al.*, 2006; Dijkstra *et al.*, 2013). In addition to the short-term increases in metabolic rate associated with activity costs during aggression, there can be longer-term energetic costs associated with social stress causing increase in metabolic rate among submissive individuals (Grantner & Taborsky, 1998; Sloman *et al.*, 2000; Killen *et al.*, 2014).

Species	Life-stage	Metabolic trait	Behaviour/performance	Relationship	Notes	Reference
Phoxinus phoxinus	juvenile	SMR	activity	none		Killen (2014)
Phoxinus phoxinus	juvenile	AS	activity	none		Killen (2014)
Salmo salar	Juvenile*	RMR	activity/dispersal	+		Robertsen *et al.* (2014)
Salmo salar	juvenile	SMR	aggression	+		Cutts *et al.* (1998)
Salmo trutta	juvenile	SMR	aggression	+	interpopulation	Lahti *et al.* (2002)
Salmo salar	juvenile	SMR	aggression	none	interpopulation	Seppänen *et al.* (2009)
Oreochromis mossambicus	adult	RMR	aggression	+	examining costs of aggressive behaviours	Ros *et al.* (2006)
Neolamprologus pulcher	adult	RMR	aggression/submission	+	examining costs of aggressive behaviours	Grantner & Taborsky (1998)
Micropterus salmoides	juvenile	SMR	angling vulnerability	+		Redpath *et al.* (2010)
Micropterus salmoides	juvenile	MMR	angling vulnerability	+		Redpath *et al.* (2010)
Micropterus salmoides	juvenile	AS	angling vulnerability	+		Redpath *et al.* (2010)
Salmo salar	juvenile	SMR	cover use	-		Finstad *et al.* (2007)
Oncorynchus mykiss	juvenile	SMR	dominance	+		McCarthy (2001)
Oncorhynchus masou	juvenile	SMR	dominance	+		Yamamoto *et al.* (1998)
Salmo salar	juvenile	SMR	dominance	+	modulated by prior residence	Cutts *et al.* (1999)
Pomacentrus amboinensis	juvenile	SMR	dominance	none		Killen *et al.* (2014)
Pomacentrus amboinensis	juvenile	AS	dominance	+		Killen *et al.* (2014)
Oncorynchus mykiss	juvenile	SMR	dominance	+		Sloat & Reeves (2014)
Salmo salar	juvenile	SMR	dominance	+		Metcalfe *et al.* (1995)
Salmo salar	juvenile	SMR	dominance	+		Reid *et al.* (2011)
Salmo salar	juvenile	RMR	dominance	+		Reid *et al.* (2012)
Oncorynchus mykiss	juvenile	SMR	early maturation	+/none	relationship in females but not males	Sloat & Reeves (2013)
Salmo salar	juvenile	SMR	early smoltification	+		McCarthy (2000)
Salmo trutta	juvenile	SMR	emergence time	-		Regnier *et al.* (2012)
Salmo salar	juvenile	RMR	emergence time	none		Vaz-Serrano *et al.* (2011)
Oncorynchus mykiss	juvenile	RMR	growth	-	oxygen uptake measured on whole tanks of fish	McKenzie *et al.* (2012)
Salmo salar	Juvenile*	RMR	growth	-		Robertsen *et al.* (2014)
Salmo salar	juvenile	SMR	growth	-/none	modulated by food availability/conspecific density	Reid *et al.* (2011)
Salmo salar	juvenile	RMR	growth	+/none	modulated by habitat complexity/food predictability	Reid *et al.* (2012)

*Metabolic rate measured at the eyed egg stage

Table A.1. Summary of documented relationships between metabolic traits (SMR = standard metabolic rate; RMR = routine metabolic rate; MMR = maximal metabolic rate; AS = aerobic scope) and behaviours or performance in several species of fish. Positive (+), negative (-), and non-existent ('none') relationships are shown for each pair of traits. Also shown is the life-stage at which the data was collected

The majority of work examining links between dominance and metabolic traits in fishes has examined juveniles competing for territories and access to food. The upper limit to metabolic rate is not really relevant in this situation since it is unlikely to be a constraint: the problem for the fish is not the rate at which food can be digested but whether or not the fish can get access to food in the first place. The costs of aggressive behaviours or an intrinsically high metabolic rate can reduce growth when food is patchily distributed (Reid *et al.*, 2011; Hoogenboom *et al.*, 2012; Reid *et al.*, 2012), and the optimal strategy may be to possess a high enough SMR to out-compete conspecifics but low enough to ensure excess energy available for growth in a particular environment. Interestingly, the position of this threshold value for SMR has been observed to increase with population density (Reid *et al.*, 2012).

Despite a general recognition that aggressive behaviour in fishes involves intense exercise (Neat *et al.*, 1998; Seebacher *et al.*, 2013), there have been few studies examining associations between MMR or AS and dominance in fishes. Killen *et al*. (2014) observed that for juvenile Ambon damselfish *Pomacentrus amboinensis* Bleeker 1868 competing for coral reef territories, dominance status was positively related to AS but not resting metabolic rate. The mechanism underlying this link between AS and aggression in the species is unclear because individuals in this species do not routinely approach maximal rates of oxygen consumption during agonistic encounters. In contrast, the increase in oxygen consumption resulting from aggressive behaviours in female adult eastern mosquito fish *Gambusia holbrooki* Girard 1859 can occupy the entire AS of an individual (Seebacher *et al.*, 2013).

Despite the volume of studies examining links between dominance and metabolic traits in fishes, there is still much more work to be done in this area. For example, most research to date has examined the role of metabolic rates during dyadic contests. Such contests may be inaccurate representations of social structures in nature, which will more likely consist of a network of interactions among individuals within a common shoal or with neighbouring territories (Sloman & Armstrong, 2002). Future work should examine the role of metabolic traits within dominance hierarchies within social networks (Croft *et al.*, 2004), or the establishment of territories within a habitat matrix with multiple interacting conspecific

A.1.4.2 Risk-associated Behaviours

The energy demand of individuals should be intimately related to the ways in which they obtain food from their environment, and potentially, the risks they are willing to take to get that food. As a result, metabolic rate could not only be related to foraging behaviours but also to traits that increase the risk of being captured by a predator, including boldness and spontaneous activity level. Individuals that display intrinsically high levels of activity may also develop an increased physiological capacity to facilitate increased rates of movement (Biro & Stamps, 2010). Correlations between MMR or AS and behaviours associated with foraging or risk-taking could therefore emerge, although to our knowledge no study has examined these potential links directly.

Consistent with observations made on other taxa, work with fishes has shown positive correlations between measures of resting metabolic rate, SMR and boldness or activity level among individuals. For example, common carp that display the riskiest behaviour have a higher resting metabolic rate when compared to those that take fewer risks (Huntingford *et al.*, 2010). Krause *et al.* (1998) demonstrated that sticklebacks that lost the most body mass during food deprivation (a proxy for metabolic energy expenditure) also showed greater reductions in the time until emergence from cover after startling when tested before and after food deprivation. For Atlantic salmon fry, individuals that had moved furthest from their nest site at the time of capture had a higher metabolic rate as measured during the egg stage (Robertsen *et al.*, 2014). Largemouth bass *Micropterus salmoides* Lacepède 1802 selected for a high vulnerability to angling – a trait which is also known to be linked to boldness and aggression (Biro & Post, 2008; Sutter *et al.*, 2012; Klefoth *et al.*, 2013) – show higher SMR and MMR as compared to individuals selected for low angling vulnerability (Redpath *et al.*, 2010). Despite the general observation that metabolic traits often correlate positively with activity, boldness, and exploratory behaviour, there are exceptions. For example, when measured in the field, brook charr *Salvelinus fontinalis* Mitchell 1814 display no link between resting metabolic rate and spontaneous activity (Farwell & McLaughlin, 2009).

There are several possible sources of discrepancies among studies in terms of the strength of correlations between metabolic traits and risk-associated behaviours. Firstly, environmental conditions may modulate the strength of correlations by altering intraspecific variation in the traits being examined (Killen *et al.*, 2013). *D. labrax,* for example, show no evidence of links between resting metabolic rate and risk-taking when

tested under benign conditions, but a positive correlation emerges among individuals after a period of food deprivation or exposure to hypoxia (Killen *et al.*, 2011; 2012a). Related to this point, it may be possible for correlations to arise depending on whether traits are measured under all environmental contexts under consideration, or if instead behaviours measured under one condition are being compared to metabolic traits measured under another (Killen *et al.*, 2013). Secondly, differences in morphological defences (e.g. spines) against predation among species may alter the relative costs of reduced foraging opportunities (Krause *et al.*, 2000), possibly generating species-specific differences in co-variation between metabolic traits and risk-taking behaviour. Thirdly, correlations between metabolism and traits such as boldness may be masked by differential responses to the stress imposed during measurement of metabolic rates by bold and shy phenotypes. For example, shy individuals may show increased oxygen consumption when measured in respirometers due to high sensitivity to handling and confinement stress (Martins *et al.*, 2011).

A.1.4.3 Foraging Ability

Most studies examining behavioural correlates between metabolic traits and foraging have focussed on the perspective of the prey, evaluating the tendency to take risks in relation to individual metabolic rates. Studies examining links to foraging success are rare, but the attack latency of predatory pike *Esox lucius* L. 1758 is positively correlated with resting ventilation rate (McGhee *et al.*, 2013). A promising area for additional research is individual variation in foraging modes. In many species, individuals will switch between actively seeking prey or lying in wait to ambush prey that come near (McLaughlin, 1989). In fishes, the tendency for an individual to choose one mode over another is affected by environmental conditions (Fausch *et al.*, 1997; Killen *et al.*, 2007), and it is possible that the environmental thresholds causing a change in strategy may vary among individuals according to their metabolic traits. Individuals with high maintenance requirements, for example, may more readily switch to an active foraging mode for increased prey intake with modest increase in the costs of locomotion (Killen *et al.*, 2007).

A.1.4.4 Habitat Selection

Given recent interest in the potential effects of AS on the geographical distribution and range of species and populations (Pörtner & Farrell, 2008; Clark *et al.*, 2013), it is surprising that more work has not been done to determine whether metabolic traits influence the habitat preferences of individuals in response to spatial or temporal variation in factors such as temperature or hypoxia. As an example of this approach, the SMR of

individual *P. phoxinus*, as measured at a single temperature, has been shown to exhibit a negative correlation with chosen temperature in a shuttle-box apparatus (Killen, 2014). It is possible that individuals with higher maintenance requirements select lower temperatures in order to increase excess energy that can be allocated to growth, or to reduce the costs of activity and digestion to increase available AS, although the extent to which energy is 'freed up' by moving to a lower temperature will depend on whether SMR drops faster with decreasing temperature than does MMR. Differences in conspecific density, predator density, food availability, or structural complexity among habitats complicate this picture (Chabot & Guénette 2013), so could cause individuals with different metabolic traits to prefer different habitats, either directly or via correlations with traits such as boldness. An example is juvenile *S. salar*, in which individuals with a lower SMR spend more time in covered areas (Finstad *et al.*, 2007b).

A.1.4.5 Links to Swimming Performance

It is commonly assumed that individuals with a higher MMR or AS should have an increased capacity for aerobic swimming performance, and this trend is generally supported when comparing among populations or treatments within a given study. However, there have been surprisingly few studies to examine links between either MMR or AS and measures of swim performance among individuals within a species. AS was found to be positively correlated with U_{crit} in cod *Gadus morhua* L. 1758 (Reidy *et al.*, 2000). Marras *et al*. (2013) observed that for *D. labrax* there was a positive correlation between MMR and the maximum attainable swimming speed during a constant acceleration test, but neither MMR or AS were correlated with any other measure of swim performance measured in that study including maximum speed during an escape response and gait transition speed (i.e. the speed at which a fish transitions from steady aerobic swimming to anaerobic "burst-and-coast" swimming). A reworking of the data presented in Killen *et al*. (2012b) reveals that for juvenile golden grey mullet *Liza aurata* Risso 1810, AS is positively correlated with gait transition speed (Pearson correlation, $r = 0.53$, $p = 0.012$), but SMR is not related to either MMR, AS, or gait transition speed.

Little is currently known of how intraspecific differences in aerobic (e.g. critical swim speed, gait transition speed) or anaerobic (e.g. fast-start escape response, maximum sprint speed) swimming performance affect the behavioural ecology of individuals. Differences in swimming ability among individuals affect their spatial positioning within a school: individuals with a relatively high AS and gait transition speed may occupy anterior

positions when the school is moving at a relatively high swim speed (Killen *et al.*, 2012b). An increased AS could allow individuals to swim at high speeds at the front of a school while simultaneously performing other physiological tasks, such as feeding or digestion. Intrinsic differences in swimming ability could be linked to intraspecific differences in migration success (Eliason *et al.*, 2011) or likelihood of escape from predation attempts (Walker *et al.*, 2005), but both of these topics require further study at the level of the individual. It is also noteworthy that individuals with a higher AS are also able to recover faster after bouts of exhaustive exercise (Marras *et al.*, 2010; Killen *et al.*, 2014). It is therefore possible that these individuals could resume normal foraging or anti-predator behaviours sooner following intense exercise (e.g. after a predator attack), but this also requires further investigation.

A.1.5 Priorities for Future Research

The increasing research focus on intraspecific variation has provided unprecedented insight into how individuals vary in the acquisition and utilisation of energy, and how these differences affect relationships between physiology and behaviour. In many respects, however, this line of research is still in its infancy. Here we outline five gaps in the current literature that we consider to be of high priority for achieving a further understanding of the ecological and evolutionary importance of individual variation in metabolic traits in fishes:

A.1.5.1 Are MMR and AS Linked to the Behaviour of Individual Animals?

Until recently studies examining co-variation in metabolic traits and behaviour in fishes have exclusively examined the role of SMR or some index of resting metabolic rate. MMR and AS could theoretically impose constraints on the simultaneous execution of aerobically-fuelled behaviours and physiological tasks, and variation in MMR or AS among individuals could therefore also be linked to differences in activity, boldness, or aggression. Knowledge of such relationships will be crucial for an integrative understanding of the adaptive value of different metabolic phenotypes (note that AS is generally more related to MMR, and hence sensitive to variation in MMR, than it is to SMR). The optimal combination of SMR, MMR, and AS in different contexts (e.g. during food deprivation, hypoxia, temperature change) is unknown. Another important area for future study is the determination of how often routine behaviours actually cause fish to approach their MMR, and whether this varies among individuals (Murchie *et al.*, 2011; Seebacher *et al.*, 2013; Killen *et al.*, 2014). Individuals that operate near MMR more

frequently could be less able to cope with factors that decrease available AS, i.e. limiting and masking factors described by Fry (1971).

A.1.5.2 How Does the Environment Modulate Links Between Metabolic Traits and Behaviours?

There is wide variation among studies with regard to the direction and magnitude of the associations between metabolic traits and behaviours. It is likely that the nature of these links are context-dependent and labile in response to changes in environmental conditions, although only a handful of studies have investigated this issue in fishes (Killen *et al.*, 2013). This is a major area requiring further study, because the degree of selection on correlated traits could vary substantially in response to subtle changes in environmental conditions, especially if factors such as temperature, oxygenation, or salinity alter the repeatability of metabolic traits. Further, depending on context, different metabolic traits may be linked to different aspects of behaviour. Food limitation, for example, may accentuate links between behaviour and SMR via differences in energy demand among individuals or effects of mass loss on behaviour. Situations which place demands on swimming performance or constrain the ability to perform physiological multi-tasking by reducing AS (e.g. exposure to hypoxia) could generate correlations between MMR or AS and behaviour.

A.1.5.3 Does the Metabolic Phenotype Influence Habitat Selection?

Although it is often assumed that metabolic traits affect the habitat preferences of fishes in response to factors such as temperature and oxygen availability (Pörtner & Knust, 2007; Pörtner & Farrell, 2008), to date this possibility has not been thoroughly studied at the level of the individual in either the laboratory or the field. Recent technological advances in telemetry have allowed the tracking of individual fish in the natural environment at temporal and spatial scales not previously possible (Cooke *et al.*, 2013; Metcalfe *et al.*, 2014 - this issue). This work has revealed intraspecific variation in habitat use and movement patterns (Kobler *et al.*, 2009), and an exciting next step in this line of research will be to determine if this variability is related to individual differences in traits such as SMR, MMR, or AS.

A.1.5.4 How do SMR, MMR, and AS Correlate Among Individuals?

Only a small number of studies have investigated correlations among SMR, MMR, and AS among individuals, and these have shown species-specific (or possibly context-specific) variation in the direction and strength of these correlations. More work on this topic could reveal fundamental links among metabolic traits that constrain plasticity or

evolutionary responses to environmental change. If an increase in MMR also requires an investment in SMR (to cover the maintenance costs of metabolic machinery), acquiring an elevated MMR may not be possible in all ecological niches or environments. Furthermore, there have been surprisingly few studies that directly examine links among either SMR, MMR, and AS and swimming performance at the individual level. Given that swimming ability could affect fitness through a variety of mechanisms (e.g. foraging ability, predator avoidance, migration, mating success and parental care), then selection on swimming performance could result in correlated selection on metabolic traits in cases where such links do indeed exist.

A.1.5.5 What is the Importance of Metabolic Traits for Behavioural Ecology Beyond the Juvenile Stage?

Studies of intraspecific diversity in metabolic traits in fishes have largely focussed on the early life-stages, examining links between metabolic traits and growth rates or competition for territory after hatching or settlement. This is in contrast to work examining intraspecific variation in metabolic traits in other taxa (e.g. mammals, birds) where work is mainly performed with adults (Rosenfeld *et al.*, 2014). An advantage of studying early life stages is that selection has had little time to reduce the variation within the population, so making it easier to detect effects of metabolic rate on fitness, but there are at least three fundamental problems with this emphasis on juvenile organisms. Firstly, the reproductive period could be an important selective bottleneck and yet we know almost nothing about how variation in SMR, MMR, or AS relate to reproductive success, mate choice and ability to provide parental care. Secondly, juveniles experience different costs and benefits associated with various behaviours as compared to adults (e.g. foraging in juveniles may come at a relatively high risk of predation) and so extrapolating links between metabolic rate and behavioural traits such as boldness or activity across life stages should be done with caution. Thirdly, juveniles are by definition growing organisms, and even though it is common practice to fast fish before measurement of SMR, prior food intake and growth trajectory can have lingering effects on behaviour, morphology, and biochemistry that may confound estimates of SMR (Killen, 2014; Rosenfeld *et al.*, 2014).

A.1.6 Summary

The extensive variation in SMR, MMR, and AS among individual fish is to some degree repeatable, but the repeatability may diminish with time, perhaps because these traits are also sensitive to a range of environmental factors including feeding regime, temperature, and oxygen availability. So far only SMR has been thoroughly studied for

links to foraging, predator avoidance, and intra-specific aggression, and more work is needed to determine whether MMR and AS also show important associations with these categories of behaviour. The available evidence suggests that the environment may affect the degree of covariation between metabolic traits and behaviour. More knowledge in this area will be especially important for understanding responses to environmental change and what suites of traits comprising metabolic phenotypes (i.e. different combinations of SMR, MMR and AS) are most advantageous under a given set of conditions.

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